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DEVELOPMENT AND EVALUATION OF AN AMBIENT VIABLE MICROBIAL AIR SAMPLER

by

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Studies were performed to evaluate the suitability of cyclone scrubber samplers and a continuously wetted substrate for detection of several types of microorganisms. The samplers were evaluated in a dynamic aerosol chamber using all-glass impingers as reference samplers. Comparison of a stainless steel and a glass cyclone scrubber sampler for detecting Bacillus subtilis var. niger spore aerosols of about 1.1-3.3 μm count median diameter showed no significant differences in their relative collection efficiencies. Based upon ease of construction, present usage, and potential availability, the glass cyclone scrubber was selected for further evaluation. This sampler showed geometric mean relative collection efficiencies, for B. subtilis var. niger spore aerosols, of 52% and 68%, depending upon the composition of the disseminating fluid. In studies using different organisms, in similar sized aerosols, this relative efficiency was 46% for Serratia marcescens, 76% for f_2 coliphage, and 92% for poliovirus type 1. During the process of aerosolization and collection, the greatest viability losses in both the reference and cyclone scrubber samplers were observed with poliovirus, followed by f_2 coliphage, and S. marcescens. It was recommended that methods used for detection of ambient viable microbial aerosols be standardized, that a selected standard sampler be evaluated under a wide range of conditions for optimization of critical parameters, and that relative microbial aerosol evaluations be performed with a reference sampler having a sensitivity similar to that of the test sampler.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report provides useful information for selecting sampling methodology to determine ambient levels of microorganisms in aerosols. A better understanding of the levels of microorganisms in aerosols will allow an evaluation of the potential health effects of such exposure and a determination of the necessity for reducing that exposure.

J. B. Lucas
Acting Director
Health Effects Research Laboratory

ABSTRACT

The purpose of this project was to enhance existing capabilities for quantitative detection of viable microorganisms in the air. The specific objectives were: (1) to review available methodologies for ambient viable microbial air sampling; (2) to design an ambient viable microbial air sampler; (3) based on a new or an existing design, to fabricate an air sampler; and (4) to evaluate the suitability of this sampler for detecting aerosols containing bacteria and viruses. In addition this study was intended to assess the applicability of various sampling methods to studies of specific environmental microbial aerosol problems.

To fulfill a need for a standard microbial large volume aerosol sampler, an air sampler design based on the principle of staged impaction was proposed. A major limitation of this sampler was that the microbial aerosol collecting substrate, consisting of a continuously wetted surface, required a considerable amount of development and optimization. Thus, to fulfill near-term needs for ambient viable microbial air sampling, the existing sampling concept of cyclone scrubbing was selected.

Studies were performed to evaluate the suitability of cyclone scrubber samplers and a continuously wetted substrate for detection of several types of microorganisms. The samplers were evaluated in a dynamic aerosol chamber using all-glass impingers as reference samplers. Comparison of a stainless steel and a glass cyclone scrubber sampler for detecting *Bacillus subtilis* var. *niger* spore aerosols of about 1.1 — 3.3 μm count median diameter showed no significant differences in their relative collection efficiencies. Consequently, based upon considerations such as ease of construction, present usage, and potential availability, the glass cyclone scrubber was selected for further evaluation. This sampler showed geometric mean relative collection efficiencies, for *B. subtilis* var. *niger* spore aerosols, of 52% and 68%, depending upon the composition of the disseminating fluid. In studies using different organisms, in similar sized aerosols, this relative efficiency was 46% for *Serratia marcescens*, 76% for f_2 coliphage, and 92% for poliovirus type 1. During the process of aerosolization and collection, the greatest viability losses in both the reference and cyclone scrubber samplers were observed with poliovirus, followed by f_2 coliphage, and *S. marcescens*.

Based on the studies, it was recommended that the methods used for the detection of low concentrations of ambient viable microbial aerosols be standardized, that a selected standard sampler be evaluated under a wide range of conditions for optimization of critical parameters, and that relative microbial aerosol evaluations be performed with a reference sampler having a sensitivity similar to that of the test sampler.

This report was submitted in fulfillment of EPA Grant No. R805864-01-1 by IIT Research Institute. The report covers the period of April 26, 1978 to February 28, 1981.

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INTRODUCTION

Project Objective

The overall purpose of this project was to enhance existing capabilities for quantitative detection of viable microorganisms in the ambient air. The specific objectives were: (1) to review available methods for ambient viable microbial air sampling; (2) to design an ambient viable microbial air sampler; (3) to fabricate an air sampler, based on a new or an existing design; and (4) to evaluate the suitability of this sampler for detecting aerosols containing bacteria and viruses. In addition, this study was intended to assess the applicability of various sampling methods to specific environmental microbial aerosol problems.

Rationale for Study

The extent of environmental exposure of human populations to infectious microbiological aerosols and the effects of such exposure on incidence of infectious disease have not been conclusively determined. Such determinations can be made, provided that reliable and sensitive methods are available to detect such aerosols or indices of exposure. Detection of indices of human exposure requires that members of a population serve as sentinels until an observable effect is demonstrated. Such human exposure can, however, be mitigated if contamination by infectious aerosols is detected early and reduced. The detection of infectious aerosols with both precision and accuracy is dependent upon the availability of adequate sampling and assay methodologies.

Numerous sampling techniques exist for detecting microbial aerosols. These techniques operate on a wide variety of principles and their use for applications requiring high sensitivity has not been standardized. Research in exerimental infectious aerobiology has involved aerosol generation in static or dynamic chambers at concentrations detectable with conventional instrumentation, usually at relatively low air sampling rates and short operational time limits. Determination of low concentrations of microbial aerosols in the ambient outdoor environment, however, requires more sensitive instrumentation. Evaluation of the risk of exposure and potential infection with source-related infectious microbial aerosols often requires an extensive sampling program. The methods used for such sampling must be sufficiently sensitive to detect such aerosols at very low concentrations. This may necessitate sampling at relatively high air flow rates for long time periods. The applicability of available air samplers to ambient environmental aerobiological studies is determined by the inherently designed capabilities. Ideally, such a sampler should have a high collection efficiency, maintain the viability of collected microorganisms without permitting growth, discriminate between respirable and non-respirable particles and collect the sample so that it can be easily assayed. In addition it should be easily sterilized, highly

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reliable, simple to operate, and capable of remote-control operation, and its cost should be such that it can be used in routine monitoring programs.

Before undertaking the task of developing of a new sampler, the available air samplers were examined to determine and select operational characteristics that would be applicable to the final design. Main emphasis was placed on air sampling devices used for detection of viable microbial aerosols. The principal aerosol collection concepts reviewed for their application to viable microbial aerosol sampling included sedimentation, filtration, impingement, precipitation, centrifugal separation, and impaction (Luckiesh, et al. 1946 (a); Luckiesh et al. 1946 (b); Fort Detrick, 1958; Wolf, et al. 1959; Batchelor, 1960; Vershigora, 1960; Kiktenko, 1965; Kiktenko, 1968; Krzysztofik, 1971; Treskunov, 1971; Fincher, 1972; Chatigny, 1978).

Experimental Approach

The research effort was performed as several consecutive tasks. The direction of each task was dependent upon the findings of previous tasks and program decisions made in consultation with the U.S. EPA Project Officer. The project tasks can be summarized as follows.

Review of Literature

A review of literature related to air samplers having application to the project objective was performed. The purpose was to determine the availability and suitability of existing instrumentation as samplers of ambient viable microbial aerosols.

Air Sampler Design

The need for a new air sampler design was based on the review of existing concepts of microbial air samplers. The design of the new air sampler was based on such criteria as estimated collection efficiency, sensitivity, reliability, ease of sterilization, viability of collected organisms, ease of sample assay, capacity for remote operation, particle size discrimination, and cost of construction and operation. The conceptual design was reviewed by several staff members of organizations designated by the U.S. EPA Project Officer.

Air Sampler Construction

Samplers based on existing designs were fabricated. The number of samplers constructed was sufficient to perform evaluation studies, and to provide additional samplers as required by the U.S. EPA Project Officer.

Air Sampler Evaluation

The sampler selected for further study was evaluated by comparing its performance in collection of viable and non-viable aerosols to that of a reference sampler. The purpose of these comparative studies was to determine the relative collection efficiency of the two samplers for the recovery of aerosols containing uranine dye, bacteria (*Bacillus subtilis* var. *niger* spores and Serratia marcescens), and viruses (f_2 coliphage and poliovirus type 1). This relative aerosol collection efficiency was expressed as:

$$CE = \frac{S}{(R_1 + R_2)/_2} \times 100 \qquad (1)$$

where

CE = relative aerosol collection efficiency

S = aerosol concentration as determined by test sampler

R_1 and R_2 = aerosol concentration as determined by paired reference samplers at pre-S position.

The aerosol slippage through the test sampler was determined by comparing the chamber aerosol concentrations detected with reference samplers located at the pre- and post-sampler positions. The pre-sampler position is upstream from the test sampler while the post-sampler position is downstream. The percent survival of bacteria and viruses during the aerosolization and sampling process was determined relative to B. subtilis var. niger spores aerosolized simultaneously with the test organism. The percent survival was calculated as follows:

$$PS = \frac{T_1}{B_1} \times \frac{B_o}{T_o} \times 100 \qquad (2)$$

where

PS = percent survival

B = B. subtilis var. niger concentration in the spray suspension (B_o) or in the aerosol sampler (B_1)

T = test organism concentration in the spray suspension (T_o) or in the aerosol sampler (T_1).

The method of sampler operation, including the sampling medium used, air sampling rates, duration of collection, and sampling fluid flow rates, was typical of that used in field studies and did not necessarily include an evaluation of multiple methods of operation. The comparative studies were performed in a dynamic aerosol chamber system using aerosol concentrations of dye or microorganisms that were detectable with both the test and reference samplers. The aerosol particle size, >1 µm count median diameter (CMD), was similar to that which might be observed in field studies of source-related ambient viable microbial aerosols.

Continuously Wetted Substrate Evaluation

A continuously wetted substrate was evaluated in a test stand device to determine its potential suitability for use in a viable microbial aerosol impacting-type sampler. The substrate was evaluated to determine:

The recovery of *B. subtilis* var. *niger* spores after collection in nutrient broth and transferring the membrane surface to nonselective nutrient agar;

Its applicability for recovery of total coliform (*Enterobacter aerogenes*), fecal coliform (*Escherichia coli*), and fecal streptococcus (*Streptococcus fecalis*) by plating onto selective media; and

The performance of the substrate-containing device compared to that of a slit sampler for recovery of *B. subtilis* and *S. marcescens*.

CONCLUSIONS

1. There is a need for standardization of methods for detecting low-level ambient viable microbial aerosols.

2. An air sampler based on the principle of staged impaction, with aerosol classification into respirable and non-respirable size ranges, may be superior to existing samplers.

3. A major limitation of this sampler design, however, is that the substrate for final microbial aerosol collection required further development and optimization prior to use in such a sampler.

4. A continuously wetted surface developed as a potential collecting substrate is suitable for collecting bacterial aerosols. Additional studies are needed to determine operating conditions optimal for survival of collected organisms on such a surface.

5. Until the collection substrate is optimized and the sampler design concept developed, an existing sampler, such as a cyclone scrubber, should be used for viable microbial aerosol studies where low concentrations are expected.

6. The existing sampling concept that demonstrates the greatest overall superiority, in terms of the criteria used for sampler evaluation, is that of cyclone scrubbing.

7. Comparison of a glass and a stainless steel cyclone scrubber showed that there was no significant difference in their relative efficiencies for collecting *B. subtilis* var. *niger* spore aerosols.

8. Further studies with the glass cyclone scrubber showed that the geometric mean relative collection efficiency for 1 to 3.5 μm CMD aerosols containing *B. subtilis* var. *niger* spores was 52% and 68%, depending upon the aerosol composition. The efficiency for *S. marcescens* was 46%, for f_2 coliphage 76%, and for poliovirus type 1 92%.

9. *S. marcescens*, f_2 coliphage, and poliovirus type 1 showed viability losses during the aerosolization — sampling process. The survival of poliovirus was less than or equal to 1% of that observed for the coliphage in samples collected with all-glass impinger reference samplers and with a glass cyclone scrubber.

10. Because of the great disparity between the air volumes sampled with the all-glass impinger and the cyclone scrubber test samplers, substantial fluctuations in relative aerosol collection efficiencies can be observed.

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RECOMMENDATIONS

1. Methods used for detecting low-level ambient viable microbial aerosols should be standardized. These methods should employ a sampler that is specifically designed for this application, and that is reliable, robust, and can be effectively sterilized by conventional methods. Until such a sampler is developed, an existing device such as a cyclone scrubber sampler, should be used.

2. The standard sampler should be evaluated under varied and controlled conditions to optimize collection efficiency by selecting appropriate collecting media for the organisms being studied and by controlling critical parameters such as fluid flow and air sampling rates.

3. These evaluations should employ a viable microbial aerosol reference sampler that operates at an air sampling rate similar to that of the device under evaluation.

MATERIALS AND METHODS

Experimental Aerosol Chamber

An aerosol sampler evaluation chamber system was designed and fabricated. The containment chamber was maintained under negative pressure and consisted of a series of three interconnecting Plexiglas chambers shown in Figure 1. Chamber A housed the equipment used to generate aerosols into a slotted diffuser tube, which opened directly into and ran the length of Chamber B. A fan (Dayton axial fan, 100 cfm, 3000 rpm) placed at the bottom of Chamber B was used to mix the aerosol; the relative humidity was determined using an Airguide dial hygrometer. The mixed aerosol was pulled into Chamber C which contained the dynamic aerosol sampler evaluation system.

The system (Figure 2) was constructed of a Staticide®-treated PVC plastic and consisted of an approximately 65 cm long and 10 cm diameter tube connected to a pre-sampler inlet manifold. Six 1.3 cm diameter aluminum sampling tubes were placed through the 90 degree bend in the manifold and positioned parallel to the airflow. The test sampler was connected to the posterior outlet of the pre-sampler inlet manifold. A post-sampler exhaust manifold, with aluminum sampling tubes positioned into the direction of air flow, was attached to the test sampler exhaust. Vacuum was supplied to the test sampler train by a blower positioned posterior to the post-sampler exhaust manifold. This blower was followed by another blower that pulled exhaust air through a HEPA filter system. The vacuum for reference and particle size analysis samplers was provided by positive displacement pumps. Air flow rates were determined with the mass flow meter (TSI model 2017) at a post- or pre-sampler location, or with a hot wire anemometer (Kurz model 441) at the pre-sampler location. Vacuum at pre- and post-sampler ports was measured in cm Hg with magnehelic gauges. Relative humidity and temperature were determined with wet- and dry-bulb thermometers.

Aerosols

Spray Suspension

The spray suspension used for aerosol generation was prepared by mixing suspensions of test organisms or other test material with suspending fluid to provide concentrations calculated to be detectable and countable in both reference and test aerosol samplers. The suspending fluid consisted of either distilled water, 5% sucrose, or 3.5 to 5% dextran solutions.

Bacteria

To assess sampler efficiency and to determine survival of vegetative microorganism, spores of *Bacillus subtilis* var. *niger* were used. These

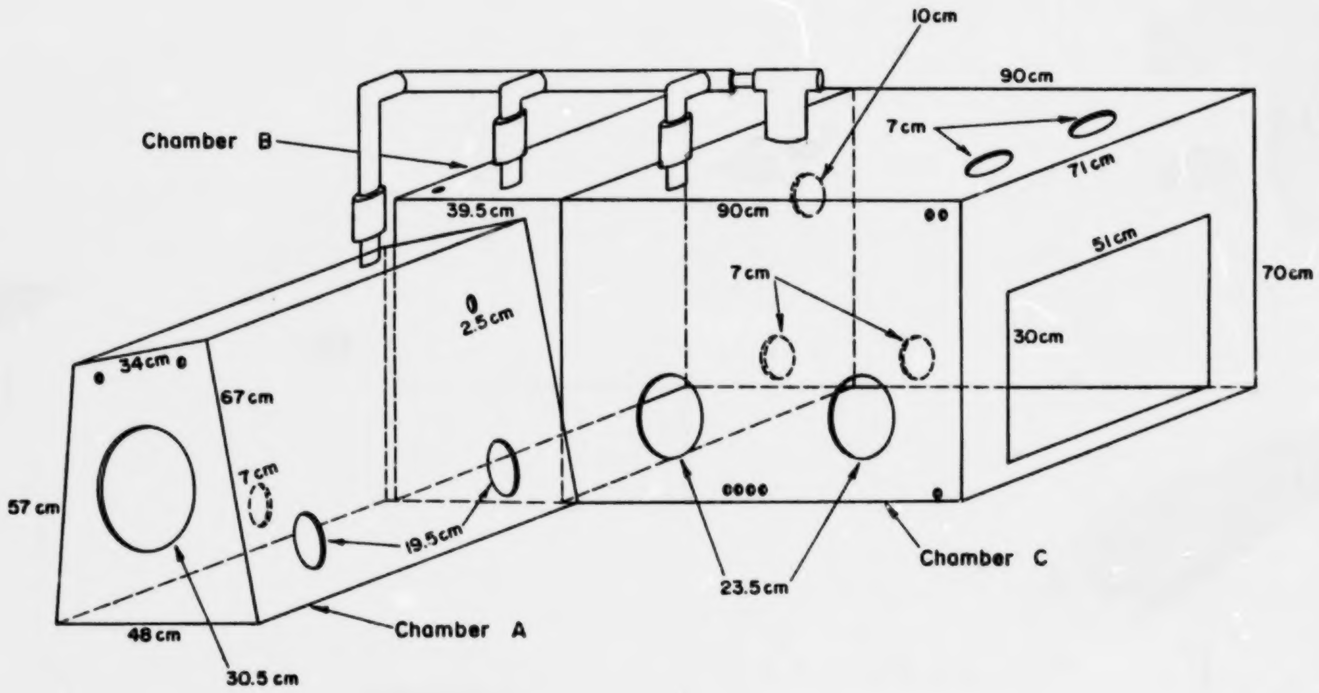
10cm
90cm
Chamber B
7cm
71cm
39.5cm
90cm
70cm
51cm
0
2.5cm
7cm
34cm
30cm
67cm
57cm
7cm
19.5cm
Chamber C
23.5cm
48cm
Chamber A
30.5cm

FIGURE 1. Aerosol sampler evaluation chamber.

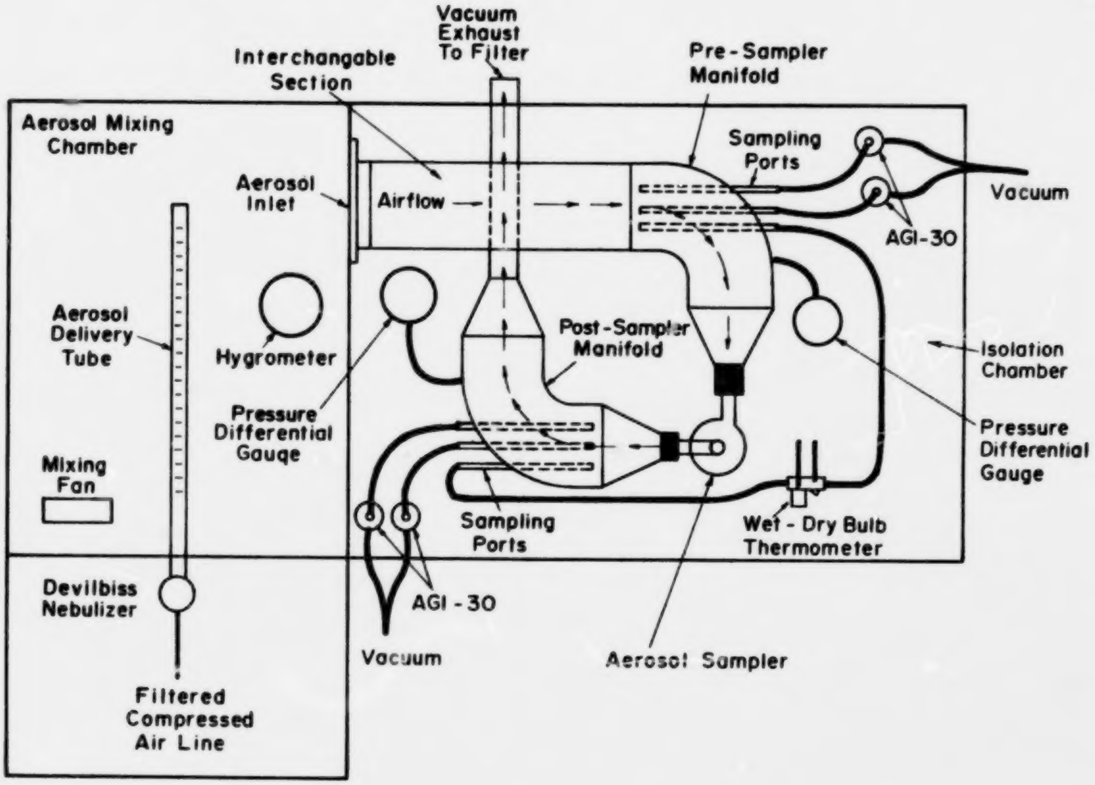
Vacuum Exhaust To Filter
Interchangable Section
Pre-Sampler Manifold
Aerosol Mixing Chamber
Sampling Ports
Vacuum
Aerosol Inlet
Airflow
AGI-30
Aerosol Delivery Tube
Hygrometer
Post-Sampler Manifold
Isolation Chamber
Pressure Differential Gauge
Pressure Differential Gauge
Mixing Fan
Sampling Ports
Wet - Dry Bulb Thermometer
Devilbiss Nebulizer
AGI - 30
Vacuum
Aerosol Sampler
Filtered Compressed Air Line

FIGURE 2. Aerosol sampler evaluation system

spores, originally obtained from the U.S. Army, Fort Detrick, MD in frozen stock, were washed in distilled water and re-suspended in pH 7.4 phosphate buffered water (PBW). Prior to use, the spore suspension was titered by plating replicate dilutions on trypticase soy agar (TSA) using a conventional spread plate technique and stored at 4°C.

Serratia marcescens ATCC No. 14756 was grown from a stock culture onto TSA plates. A typical colony was picked, transferred to a flask of trypticase soy broth (TSB), and incubated with continuous agitation at 35°C for 24 hrs. The growth was pelleted by centrifugation (950 X g at 4°C for 30 min), washed, and resuspended in PBW. Cultures of Streptococcus faecalis (ATCC No. 6057), Escherichia coli (ATCC No. 15669), and Enterobacter aerogenes (ATCC No. 13048) were prepared in a similar manner.

Coliphage

A 4-hr culture of E. coli K12HfrD (ATCC No. 15669) grown in phage assay broth (PAB), consisting of 8.0 g nutrient broth; 5.0 g NaCl; 0.20 g $MgSO_4 \cdot 7H_2O$; 0.05 g $MnSO_4 \cdot H_2O$; and 0.15 g $CaCl_2$ per 100 ml H_2O was inoculated with a diluted stock of f_2 coliphage and incubated on a shaker at 35°C for 18 hr. After the addition of 5% (v/v) chloroform, the bacteria-virus suspension was mixed by shaking and held at 4°C for 5 hr. The suspension was then centrifuged at 950 x g at 4°C for 30 min, and the supernatant was titered by a soft agar overlay plaque assay procedure and stored at 4°C until used.

Poliovirus

Buffalo Green Monkey Kidney (BGMK) cell monolayers (Grand Island Biologicals, Inc. or HEM Research, Inc.) were produced in roller bottles in Minimum Essential Medium (MEM) containing 10% fetal calf serum (FCS), 10 mM HEPES buffer, 50 μg/ml Gentamicin, and 2.5 μg/ml Fungizone. The monolayers were washed three times with Hanks Balanced Salts Solution (HBSS), inoculated with 1 ml of diluted poliovirus type 1 LSC 2ab stock (approximately 1×10^2 pfu/ml) and incubated on a roller apparatus for 1 hr at 37°C. The MEM, containing 2% heat-inactivated FCS (HIFCS), was added to the inoculated cells and the roller bottles were incubated at 37°C until the cell monolayer began to slough off (approximately 48 hr). After addition of 5% (v/v) chloroform, the cell-virus suspension was mixed by shaking and held at 4°C for 5 hr. The cell suspension was centrifuged at 950 x g at 4°C for 30 min, and the supernatant was aliquoted, titered by plaque assay, and stored at —70°C until used.

Uranine Dye

Uranine dye solutions were prepared to a concentration of 0.2 g/ml in either distilled water or a 5% sucrose solution. Appropriate dilutions of the solution were used to calibrate the Coleman photofluorometer (Model 12 C) used for sample analysis.

Aerosol Generation

DeVilbiss No. 40 nebulizers were loaded with 10 ml spray suspension and, using 20 psig filtered room air, test aerosols were continuously generated throughout the duration of each experimental trial and delivered to the experimental system as described above. The relative humidity was monitored in the mixing chamber and the temperature ranged from 24° to 27°C. Upon completion of each experimental trial, the volume of liquid remaining in the nebulizer was measured and recorded.

Air Samplers

Reference Samplers

AGI-30 (Figure 3), containing 20 ml of sampling fluid (distilled water or 25% TSB), were employed as reference samplers. Two AGI-30 were operated in the pre-experimental sampler position and, after air moving equipment modifications, two samplers were operated in the post-experimental sampler position. The air flow rate of each sampler was calibrated with a mass flow meter (Kurz Model No. 543) and averaged 0.0125 m^3/min. The AGI samplers were connected to aluminum sampling ports of 1.3 cm I.D. positioned horizontally within the dynamic chamber and directed into the moving aerosol. The linear velocity of the aerosol through the sampling port during individual sampling trials was 158 cm/sec (Table 3). This velocity was very near that of the chamber aerosol when the airflow rate was 0.8 m^3/min.

Paired 2 min samples were taken at each location at the beginning, middle, and end of each experimental trial. After collection, the AGI samplers were placed on ice and the sampling fluid volume was adjusted to 20 ml with distilled water.

Experimental Samplers

Glass Cyclone Scrubber

The glass cyclone scrubbers used in the studies were based on the general design described by Decker, et al. (1969) and on prototype samplers provided by I. Abelow, U.S. Army, Edgewood Arsenal, MD. (Figure 4.) The sampler inlet arm was positioned perpendicular to the body of the sampler. During collection, approximately 2 ml/min collecting fluid was pumped using either a syringe (Sage Instruments Model 220) or a peristaltic pump (Sage Orion Model 375A). The fluid was pumped through a needle into the throat of the inlet arm and aspirated into a fine mist (Figure 5). The mist droplets were thrown out of the airstream by spirally moving air and tangentially impacted onto the wall of the scrubber. These droplets then coalesced creating a film that covered the wall and moved helically toward the lower apex of the scrubber where it was collected by the lower pressure created by the sampler vacuum. The collecting vessel was a serum stoppered bottle with two inserted 18 gauge needles, for sample collection and air venting, attached to the lower apex of the scrubber using a "Y"-shaped tygon tubing connecter. A 30 min scrubber sampling tine was selected to reduce sampling error caused by the slow sampler

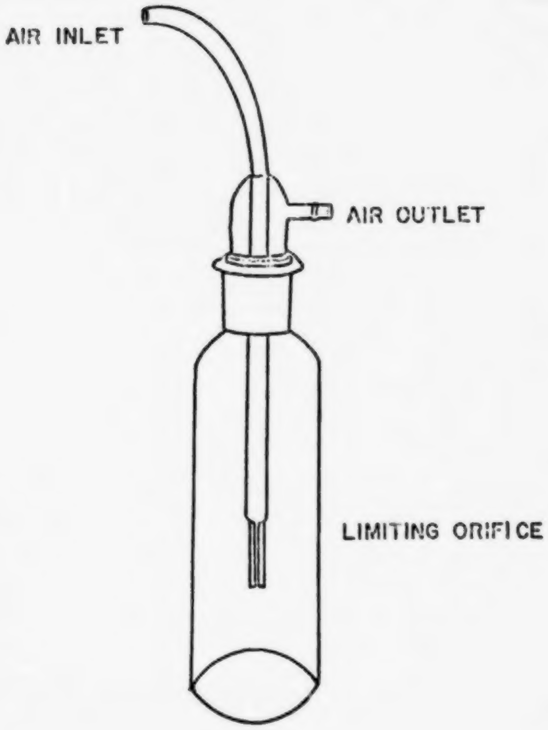
AIR INLET
AIR OUTLET
LIMITING ORIFICE

FIGURE 3. All-glass impinger.

TABLE 1. LINEAR AIR VELOCITY THROUGHOUT TEST SYSTEM AT VARYING FLOW RATES

Air Flow	Linear Air Velocity (cm/sec)		
Rate (m^3/min)	Dynamic Chamber	AGI	Cyclone Scrubber
1.0	208	158	5300
0.9	187	158	4780
0.8	166	158	4240
0.7	143	158	3710
0.6	122	158	3180

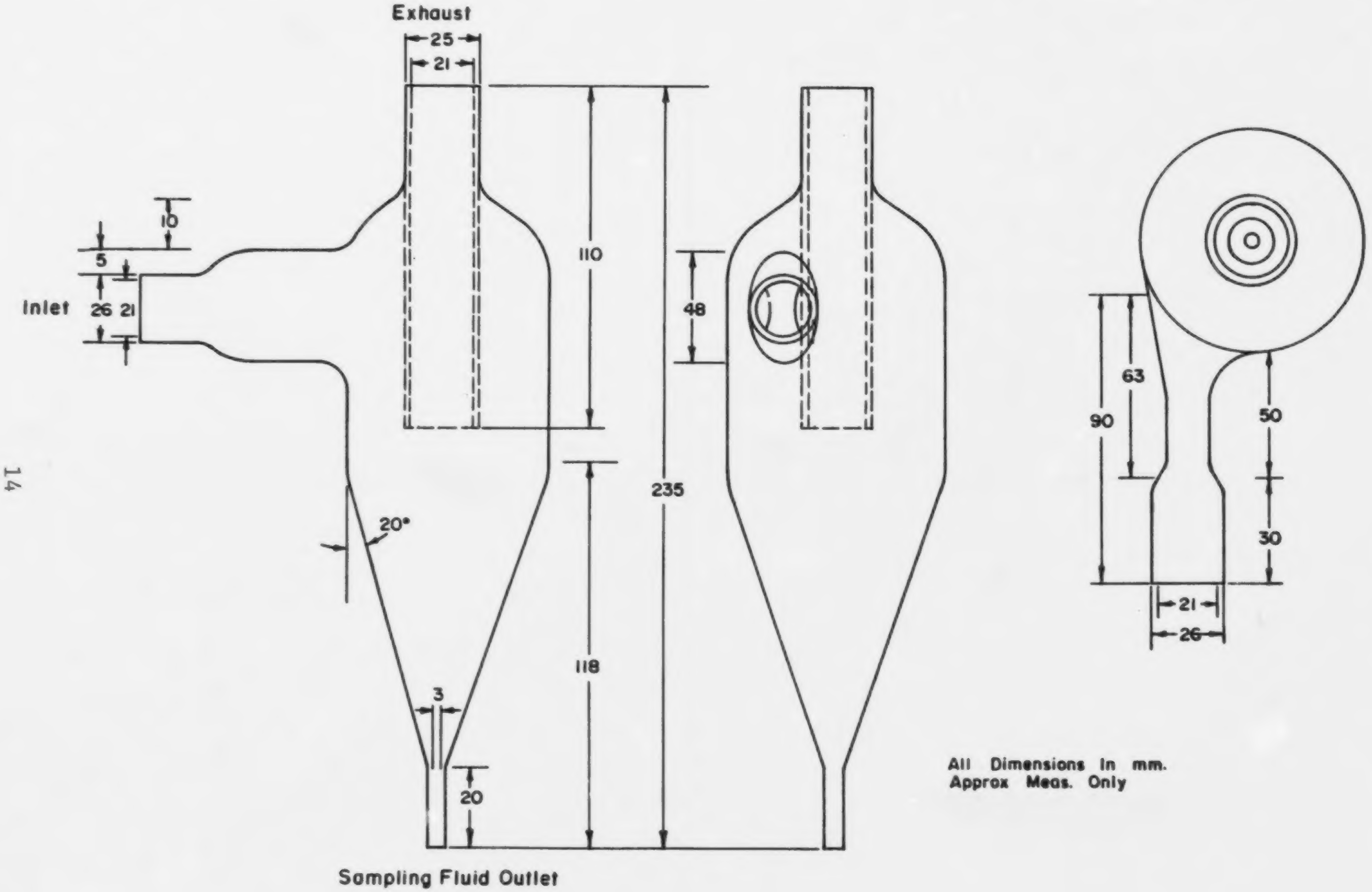
Exhaust
25
21
Inlet
10
5
26
21
110
118
235
48
20°
3
20
Sampling Fluid Outlet
63
90
50
30
21
26
All Dimensions In mm.
Approx Meas. Only

FIGURE 4. Glass cyclone scrubber aerosol sampler.

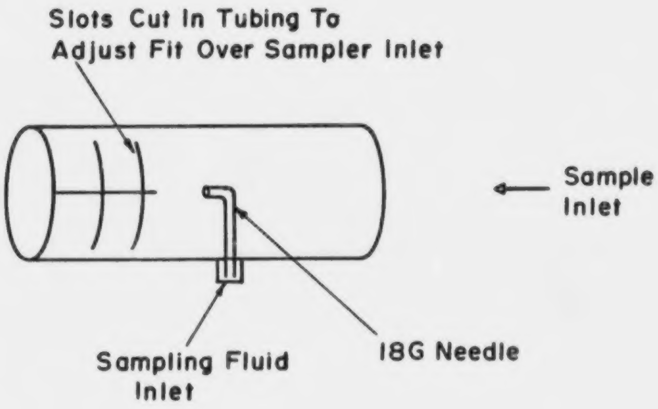
Slots Cut In Tubing To Adjust Fit Over Sampler Inlet
Sample Inlet
Sampling Fluid Inlet
18G Needle

FIGURE 5. Sampling fluid inlet fitting.

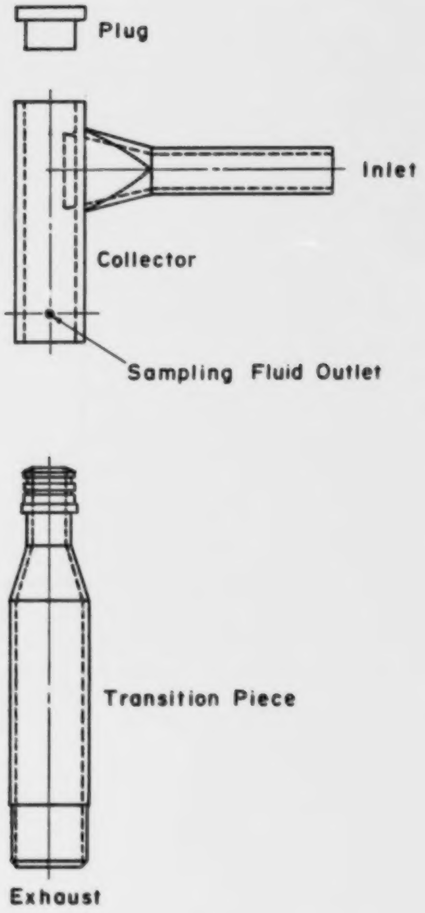
Plug
Inlet
Collector
Sampling Fluid Outlet
Transition Piece
Exhaust

FIGURE 6. Stainless steel cyclone scrubber aerosol sampler.

response time and to more closely simulate sampling conditions required for detecting microbial aerosols in low concentrations.

Stainless Steel Cyclone Scrubber

The stainless steel cyclone scrubber was based on the sampler design specifications from the Defense Research Establishment, Suffield, Alberta, Canada, and described by White, et al. (1975). The inlet of the sampler was positioned perpendicular to the cylindrical collecter (Figure 6). The point of inlet-to-collecter attachment was positioned off-center to promote a helical movement of the air toward the exhaust. A sampling fluid delivery system, similar to that shown in Figure 5, was attached to the inlet for the sampling fluid delivery via a screw-driven or peristaltic pump. As the fluid was delivered into the sampler inlet at about 2 ml/min, it was aspirated into a fine mist that impacted onto the wall of the collecter where it moved helically toward the fluid outlet. The sampling fluid was aspirated through the outlet into a serum-stoppered bottle. In contrast to the glass scrubber, an additional vacuum source (approximately 35 cm H_2O), supplied by a positive displacement pump, was required for sampling fluid collection. The air sampling duration was 30 min.

Samplers for Aerosol Particle Size Determination

Preliminary trials for particle size determinations were performed using three straight-necked AGI samplers. The AGI samplers were fitted with single-stage preimpactors having particle size cut-off classifications of 1.0, 3.0, and 5.0 µm mass median diameter (MMD) for uranine dye aerosols (Malligo and Idoine, 1964). That is, 50% of the aerosol mass was below the specified cut-off size. The portion of aerosol collected in each AGI, considered to represent particles that were smaller than the stated cut-off size of the preimpactor, was assayed during uranine dye aerosol studies. Studies with uranine dye aerosols showed, however, that the particle size was <1.0 µm MMD and could, therefore, not be classified by this procedure.

Consequently, aerosol particles were classified into general size ranges by light scattering detection with either a Royco Model 225 particle size analyzer or Andersen six-stage impactors (Figure 7). The Royco particle size analyzer was used to classify particles into 1.4, 3.0, and 5.0 µm count median diameter (CMD) size ranges, without regard to the presence of viable organisms, with 50% of the aerosol particles before the specified cut-off size. Sampling was performed at 0.003 m^3/min and duplicate 1 min average counts of the aerosol were obtained for each size classification. Particle size classification with the Andersen sampler was based on the enumeration of B. subtilis var. niger spores or S. marcescens collected from the aerosol. The relatively high concentrations of microorganisms employed in this study did not permit direct counts on trypticase soy agar from the Andersen sampler stages. A dilution and assay procedure was used whereby 15% gelatin was added to trypticase soy broth. A 27 ml quantity of the medium was poured in special glass plates, permitted to solidify at 3° to 4°C, and loaded into Andersen samplers. For each trial, paired Andersen samplers were attached to sampling ports of the dynamic aerosol chamber. Sampling was performed for 6 min during aerosol dissemination, the plates were removed, liquefied at 37°C, and decanted.

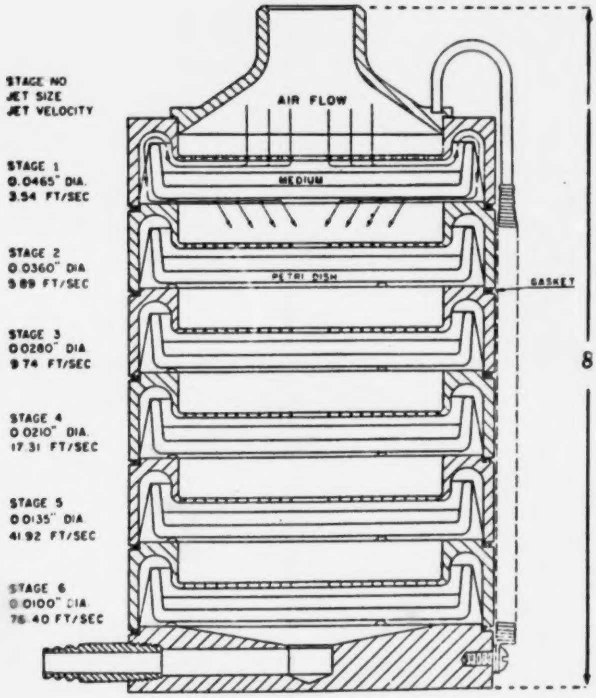
STAGE NO
JET SIZE
JET VELOCITY
AIR FLOW
STAGE 1
0.0465" DIA.
3.54 FT/SEC
MEDIUM
STAGE 2
0.0360" DIA.
5.89 FT/SEC
PETRI DISH
GASKET
STAGE 3
0.0280" DIA.
9.74 FT/SEC
8"
STAGE 4
0.0210" DIA.
17.31 FT/SEC
STAGE 5
0.0135" DIA.
41.92 FT/SEC
STAGE 6
0.0100" DIA.
76.40 FT/SEC

FIGURE 7. Andersen multistaged impactor sampler.

(Reprinted from Andersen, 1958
by Permission of American Society
for Microbiology.)

Serial dilutions were prepared in phosphate buffered water and 0.1 ml aliquots were assayed in duplicate on trypticase soy agar (TSA) by the conventional spread plate technique. After incubation at 35°C for 24 hr, the colonies were counted, the counts from paired samplers averaged, and the CMD and aerosol particle size range were determined.

Continuously Wetted Substrate

Several collecting surface concepts for use in impactor-type samplers were considered for further evaluation. The selected concept was that of a continuously wetted substrate that could be used to collect the aerosols and permit simple assay of a wide variety of microorganisms.

To evaluate this concept, a test stand was constructed on the principle of a slit impactor sampler. The prototype of the test stand is shown in Figure 8. The slit opening was 38.5 mm X 0.2 mm and was positioned 3.0 mm above the aerosol collecting surface. This collecting surface consisted of a 47 mm diameter membrane filter placed on an absorbant pad saturated with a non-selective medium (25% TSB was used in this study). Prior to each set of samples, the device was autoclaved and between individual samples was swabbed with 70% ethanol. During the air sampling, the sampling surface carrier rotated at one revolution per hour and the water lost through evaporation was continuously replaced using a screw-driven syringe pump. Air was collected at 0.028 m^3/min using a calibrated critical orifice and a positive displacement pump. After sampling, the membrane filter was removed from the absorbant pad and was placed on either a non-selective or a selective medium to enumerate the bacteria-containing particles. The membrane filter could also be placed in a suitable filter holder for elution of bacteria or viruses for subsequent assay.

Slit Sampler

The slit sampler (Matteson/Garvin Model 200) (Figure 9) was used as a reference sampler for comparative studies of the prototype wetted substrate test stand. The critical slit dimensions and slit-to-surface distance were similar to those of the test stand described above. The collecting substrate in the slit sampler consisted, however, of TSA in 150 mm diameter plastic petri dishes. Air was sampled at 0.028 m^3/min and the sampling surface rotation was one revolution per hour. Prior to sampling, the sample inlet slit and housing were swabbed with 70% ethanol.

Assay and Enumeration

Bacteria

Tenfold dilutions of samples collected in the AGI or cyclone scrubber samplers were assayed in 0.1 ml volumes on TSA by conventional spread agar plate techniques. When both *B. subtilis* var. *niger* and *S. marcescens* were studied, as in vegetative bacteria survival studies, assays were performed on TSA containing 0.25 μg/ml potassium tellurite or 0.5 μg/ml brilliant green. Samples from slit and agar-containing Andersen samplers were incubated and counted. Samples from the wetted surface test stand were assayed by trans-

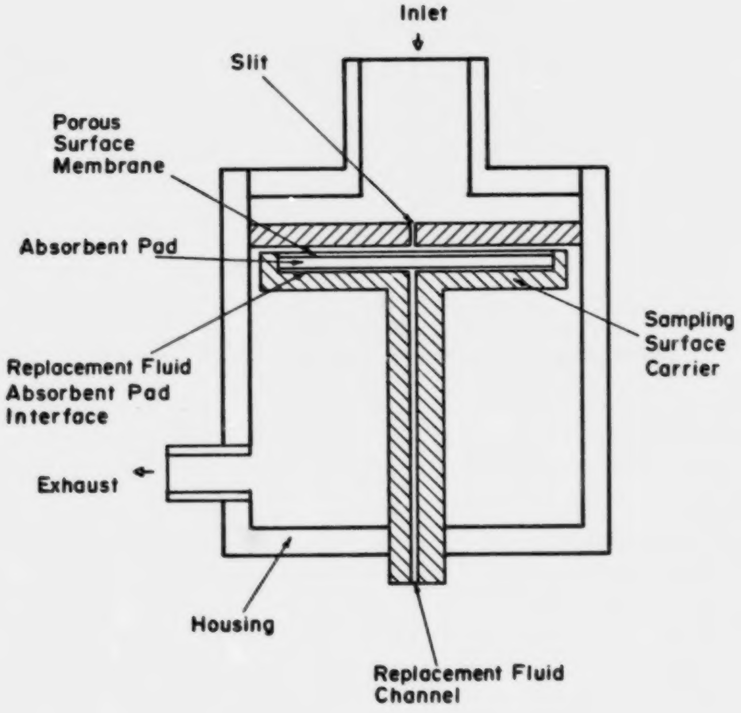
Inlet
Slit
Porous Surface Membrane
Absorbent Pad
Sampling Surface Carrier
Replacement Fluid Absorbent Pad Interface
Exhaust
Housing
Replacement Fluid Channel

FIGURE 8. Prototype wetted surface test stand.

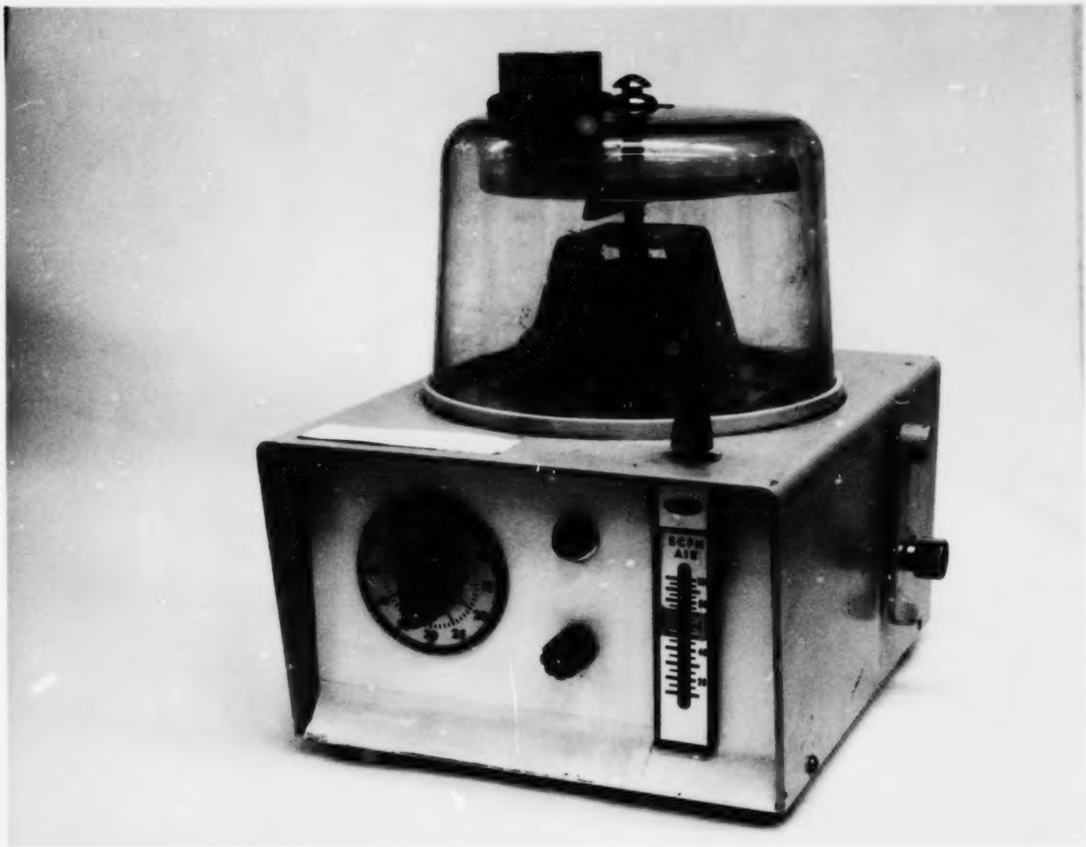

FIGURE 9. Slit sampler.

ferring the bacteria-laden membrane onto either an agar surface or onto a liquid medium-saturated 47 mm diameter absorbant pad. The media used for different organisms were as follows:

- Total aerobic bacteria — TSA (BBL)
- *B. subtilis* var. *niger* — TSA (BBL)
- *S. marcescens* — TSA
- *S. facalis* — KF Streptococcus agar (Difco)
- *E. coli* — m-FC broth (Difco)
- *E. aerogenes* — m-Endo broth (Difco)

Samples for these bacteria were assayed according to *Standard Methods*, 14th Ed. (1975). *E. coli* was incubated at 44.5 ± 0.2°C in a waterbath incubator, while the remaining organisms were incubated in an air incubator at 35 ± 0.5°C. *S. facalis* was incubated for 48 hr while the remaining organisms were incubated *for 24* hr. Colonies were counted, using the positive hole correction (Andersen, 1958), and recorded as colony-forming units (cfu) per sample volume.

Coliphage

An aliquot of sampling fluid was filtered through a HIFCS-pretreated 0.22 or 0.45 µm membrane filter of 25 mm diameter. Tenfold dilutions were prepared in PBW and assays were performed on *E. coli* K12HfrD cells in quadruplicate by a soft-agar overlay technique. For this assay, melted 5 ml volumes of phage assay broth (PAB) with 0.7% agar, were held at 45°C, inoculated with 0.1 ml 4-hr culture of *E. coli* K12HfrD and 0.1 ml of test sample, mixed by vortex agitation, and poured onto a hardened base of PAB with 1.5% agar. The plates were permitted to harden, inverted, incubated at 37°C for 24 hr, and counted for plaque forming units (pfu).

Poliovirus

Poliovirus dilutions were prepared in HBSS containing 10 mM HEPES buffer. Plaque assays were performed in BGMK cells in monolayered plastic tissue culture flasks. The cells were washed twice with HBSS containing HEPES buffer and inoculated with 1.0 ml of appropriate assay dilutions. The flasks were then incubated at 37°C for 1 hr before addition of MEM overlay containing 1% of 50% $MgCl_2 \cdot 6H_2O$, 2% HIFCS, 50 µg/ml Fungizone, 4% of a 1:1000 neutral red solution and 1.5% Difco Bacto agar. The flasks were inverted and incubated in the dark at 37°C. Plaques were marked daily, counted, and recorded as pfu per ml of sample.

Poliovirus titrations for virus aggregation studies were performed on BGMK cells grown as described above and monolayered in flat-bottom 96 well microtiter plates. The cells were washed twice with Earles Balanced Salts Solution and the samples diluted in MEM were inoculated in 0.1 ml volumes at seven replicates per log dilution. After the addition of 0.1 ml of MEM to each well, the plates were incubated at 37°C in a 5% CO_2 atmosphere and daily observed for CPE. The fifty percent tissue culture infections dose ($TCID_{50}$) was calculated for each sample according to the Reed-Muench method.

RESULTS AND DISCUSSION

Development of a Proposed Ambient Viable Microbial Air Sampler Design

The review of ambient viable microbial aerosol-related studies (see Appendix) indicated that numerous samplers have been developed or applied to such studies. Although some progress has been made to standardize low-volume samplers, similar progress has not been made with regard to higher volume samplers capable of detecting relatively low concentrations of microbial aerosols. Some of the limitations of available instruments used for detecting low-level microbial aerosols have been reviewed. Based on this review, a need for a sampler designed specifically for detecting low-level source-related microbial aerosols, but with fewer limitations than existing devices, was recognized. Consequently, a new sampler design was put forward which was based on the aerosol collection principle of impaction and permitted aerosol classification into respirable and non-respirable fractions.

Impaction refers to the process of imparting sufficient inertia to a particle to cause it to leave an airstream, following a rapid directional change, and to strike a gaseous, liquid, or solid medium. Impaction is dependent upon particle inertia which is influenced by such parameters as particle size, density and gas velocity. The jet-to-impaction target distance is critical to collection efficiency. In the past air sampling devices such as the Andersen and slit samplers have effectively employed the impaction concept for collection of airborne bacteria.

Hatch (1961) classified particles smaller than 5 µm as respirable and noted that 1 to 2 µm particles achieve greater deposition in the "lobular" region of the lung than 0.25 to 0.5 µm particles. Based upon this definition, the 1 to 5µm microorganism-containing particles are of greatest interest from a public health point of view.

A sampler was designed to collect microorganism-containing particles with predictable efficiency, and to classify them into 1 to 5 µm and $\geq$ 5 µm size ranges while retaining the viability of the microorganisms. The viable aerosol stage virtual impactor (VASVI) (Figure 10), has distinct advantages to those of any existing sampler and is based on the concept of staged impaction. The circular inlet is directed into the wind by a vane, and the particles are accelerated to a sufficiently high velocity to impact into a smaller airstream, while the very small particles and the air are exhausted. The particles are then classified into respirable and nonrespirable sizes by using a second stage for airstream impaction and each particle size is collected onto a surface by impaction. At each stage, particles are accelerated to achieve sufficient inertia to be impacted into a slower moving airstream or onto a surface, while smaller particles are carried with the larger airflow. The upper size limit of these concentrated particles is determined by inlet losses

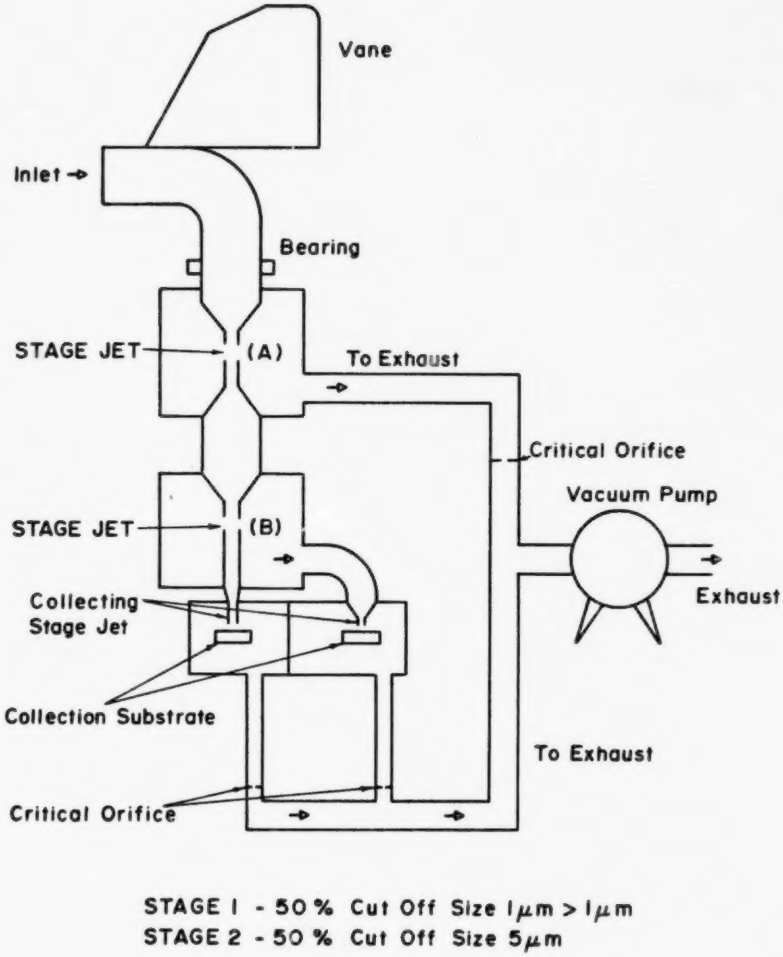
Vane
Inlet
Bearing
STAGE JET
(A)
To Exhaust
Critical Orifice
Vacuum Pump
STAGE JET
(B)
Collecting Stage Jet
Exhaust
Collection Substrate
To Exhaust
Critical Orifice
STAGE 1 - 50 % Cut Off Size 1μm > 1μm
STAGE 2 - 50 % Cut Off Size 5μm

FIGURE 10. Viable aerosol staged virtual impactor.

and is influenced by the inlet air velocity, diameter of the inlet tube, and the radius of the inlet curvature. The final volume of air into which the particles are concentrated can be relatively small. For example, if the inlet airflow rate is 1 m^3/min at an air velocity of 3.6 m/sec through a 7.75 cm diameter inlet and the particles are concentrated into 10% of that air flow in the first stage, all 1 μm particles would be in 0.1 m^3/min of air.

The first sampler stage is based on an assumed collection efficiency of 50% for 1 μm diameter particles of unit density based on impaction theory. Assuming a Reynolds number of 9000, 108 radially oriented jets of 0.166 cm diameter are required in the first stage. Particles greater than the specified aerodynamic size are impacted into a relatively low flow airstream (approximately 0.1 m^3/min) while smaller particles are carried and exhausted with the high flow airstream (approximately 0.9 m^3/min). The low air flow is carried to the second stage through two jets of 0.83 cm diameter where 5 μm diameter particles are impacted with 80% efficiency into a lower air flow (approximately 0.09 m^3/min). Particles of both sizes are collected by conventional impaction onto an autoclavable moving collection substrate. Larger particles are collected by acceleration through a 0.1 cm jet (80% efficiency for 5 μm diameter particles) while smaller particles are accelerated through a 0.18 cm jet (50% efficiency for 1 μm diameter particles). These airflow rates, which may also be achieved by using slits or other critical orifices, are within ranges attainable with principles now widely used in aerobiology. The robustness of the system requires the use of a reliable and easily sterilizable collection substrate.

The design of this air sampler was based on experience, a review of existing concepts and awareness of the need for a simple and robust device for field detection of microbial aerosols. The proposed design concepts were reviewed by I. Abelow and S. Mumford, U.S. Army, Aberdeen Proving Ground, MD, by S. Schaub, E. Larson, and H. Bausum, U.S. Army, Fort Detrick, MD, and by the U.S. EPA Project Officer.

Selection of Sampler Design for Fabrication and Further Study

Several sampling concepts and devices were compared as potential alternatives to the viable aerosol staged virtual impacter for fabrication and further evaluation. Selected widely used samplers based on several aerosol collection concepts were ranked with regard to their potential suitability as ambient viable microbial air samplers. These rankings were performed by selecting ten criteria that were weighted with respect to their relative importance to ambient viable aerosol sampler selection. The criteria and their assigned weighting factors in decreasing order of importance were as follows:

collection efficiency (10); relatively efficient particle collection.

sampling sensitivity (9); collect particles from relatively large volumes of air.

reliability (8); dependable for extended field operation.

ease of sterilization (7); sterilizable by conventional means, eg. autoclavable.

maintenance of viability (6); degree of microorganism survival after collection.

ease of sample assay (5); easy to assay for variety of microorganisms including viruses.

ease of operation (4); simple operation requiring minimal training of personnel.

remote operation capability (3); ability to operate unattended for extended time period.

particle size discrimination (2); classification of respirable and non-respirable particles.

cost (1); relative affordability for routine monitoring programs.

Each sampler was ranked as high, moderate, or low with respect to each parameter, and assigned a relative ranking value of 1, 2, or 3, respectively. These rankings were multiplied by the weighting factor given each criterion. The weighted rankings of all criteria were added for each sampler. In the final analysis, the most desirable devices were those with the lowest total numbers. The lowest rankings were achieved by the cyclone scrubber samplers while the highest rankings were observed with the large-volume electrostatic precipitator and multislit impinger samplers (MSI) (Table 2).

Gravitational sedimentation is neither quantitative nor very sensitive. Such a concept depends upon the settling velocity of airborne particles and is much more efficient for larger particles than for small ones. While electrostatic precipitation, which is used in the LVAS and LEAP samplers can be used to efficiently collect airborne particulates, use of high voltage equipment reduces robustness and increases the likelihood of electrical arcing in these samplers. These electrostatic precipitator samplers and the MSI have an asset in that they operate at high air sampling rates using a liquid film. This liquid film, however, must be continuously and evenly spread over a rotating disc. Slight maladjustments of the fluid pick-up arms or spreaders can cause sampling fluid losses. Other difficulties observed with these samplers are that they are generally incompatible with conventional sterilization procedures, require constant attention to maintain a completely wetted disc, require technical skill for operation, are difficult to handle and frequently break down in the field. Filtration is an efficient means of removing particulates from an airstream. Since many organisms rapidly lose viability upon desiccation, this concept is most applicable for collection of highly resistant microbial aerosols. Although impingers (e.g. AGI) and impactors (e.g. Andersen or slit samplers) are useful for many applications, they sample at relatively low air flow rates, reducing their sensitivity for detecting low-level aerosols. Based on these relative rankings, the cyclone scrubber is the most desirable existing sampler alternative to the viable aerosol staged

TABLE 2. RELATIVE RANKING[a] OF AMBIENT VIABLE MICROBIAL AIR SAMPLERS

Weight Factor	Criteria[b]	Sedimentation	Filtration	Impingement		Electrostatic Precipitation		Cyclone Scrubbing		Impaction	
		Settling Plate	Membrane Filter	AGI	MSI	LVAS	LEAP	Glass	Steel	Andersen	Slit
10	Efficient Collection	30	10	10	10	10	10	10	10	10	10
9	Sensitive	27	18	27	9	9	9	9	9	18	18
8	Reliable	8	8	8	16	16	16	8	8	8	8
7	Easily Sterilized	7	7	7	21	21	21	7	7	7	7
6	Maintain Viability	6	18	6	6	6	6	6	6	6	6
5	Assay Ease and Versatility	10	10	5	5	5	5	5	5	10	10
4	Simple Operation	4	4	4	12	12	12	8	8	4	4
3	Remote Operation	3	6	9	9	9	9	6	6	6	6
2	Size Discrimination	6	6	6	6	6	6	6	6	2	6
1	Cost	1	2	1	3	3	3	1	2	3	2
		102	89	75	97	97	97	66	67	74	77

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[a] Lowest sums of ranks indicates most desirable overall characteristics.

[b] Each weight factor is multiplied by the rating number based on judged sampler characteristics for specific criteria: 1 = desirable; 2 = neutral; 3 = undesirable.

virtual impactor. To determine whether the new sampler design or a cyclone scrubber sampler should be fabricated and evaluated, both devices were compared on the basis of their advantages and limitations as shown in Table 3.

After assessment of the advantages and disadvantages, it was determined that the development and evaluation of a new sampler design should reflect the long-term U.S. EPA research goals whereas the evaluation of an existing sampler, i.e. cyclone scrubber sampler, should be the near-term research goal. A compromise between satisfying short-term needs and preparing for potential long-range microbial aerosol sampling requirements was selected. It was determined that a collecting substrate with potential application to a sampler such as the viable aerosol staged virtual impactor should be developed and evaluated while an existing cyclone scrubber sampler should be evaluated, using viable microbial aerosols, to determine its applicability for ongoing as well as near-future studies.

The selection of a suitable substrate was essential to attain optimal collection efficiency and maintenance of viability in the viable aerosol staged virtual impactor. A suitable substrate should: (1) permit aerosol collection onto a moist continually wetted surface; (2) be easy to sterilize; (3) permit easy assay for both bacteria and viruses (hence collection into a relatively small fluid volume); and (4) support viability during the sampling process. The use of reliable pumping systems having minimum likelihood of contamination and mechanical breakdown is desirable. Consequently, an aerosol collecting substrate consisting of a continually wetted porous membrane impaction surface was subjected to preliminary evaluation.

Continuously Wetted Substrate

The membrane of the collecting substrate, which was preinoculated with 25% TSB, was positioned beneath the slit of the test stand sampler and was continuously wetted with distilled water using a screw-driven syringe pump. To determine the effect of varying the rate at which the substrate was wetted, air samples were taken in empty animal holding rooms. A conventional slit sampler, using TSA medium in 150 cm diameter plates, was placed in close proximity to the wetted substrate test stand. Both samplers were operated at 0.028 m^3/min air sampling rate and at 0.5 collecting substrate revolutions per hour. The water flow rate in the wetted substrate test stand was varied from 0.1 to 0.3 ml/min. The ratio of the total aerobic bacterial aerosol concentration determined with the wetted substrate test stand to that with the slit sampler was determined. As shown in Table 4, this ratio varied considerably at each test stand fluid flow rate but the greatest variation was observed at the 0.1 ml/min rate. These ratio variations may be partially attributed to the detection of unevenly distributed aerosols within the animal holding rooms. With these variations, however, this overall ratio was lower at water flow rates of 0.1 and 0.2 ml/min, but exceeded 1.5 at a 0.3 ml/min water flow rate.

Similar studies in an aerosol chamber showed (Table 5) that the wetted substrate test sampler recovered more viable *B. subtilis* var. *niger* spore-containing particles (geometric mean ratio of 1.0% to 1.9%) but fewer viable *S. marcescens*-containing particles (geometric mean ratio of .19% to .69%) than

TABLE 3. COMPARISON OF LARGE VOLUME CYCLONE SCRUBBERS AND A VIABLE AEROSOL STAGED VIRTUAL IMPACTOR AS AMBIENT VIABLE MICROBIAL AIR SAMPLERS

Large Volume Cyclone Scrubber

Advantages

1. Device is relatively inexpensive to construct.
2. Can be autoclaved to ascertain sterility.
3. Relative efficiency has been evaluated in a limited number of available studies.
4. Can operate for long period with attention.
5. Efficiency similar to LVAS and about 92% compared to impactor (reported in Errington & Powell, 1969)
6. May (1973) preferred the Aerojet-General all glass spray-wash cyclone (Decker et al 1969) and the simple liquid scrubber (Buchanan et al 1972) over the Litton LVAS and the ERC MSI on the basis that the latter two are bulky, complicated, and very expensive.
7. Glass cyclone (British) slightly better than DRES scrubber for collecting T-1 and MS-2 coliphage
8. Cyclone proved reliable in use (Errington- Private communication)
9. No small orifices which may be subject to clogging (except feed tube)

Disadvantages

1. Glass-blown version (British) has variations in fluid tolerable fluid flow rates between samplers (e.g. two scrubbers made from same drawings had different flow rates at a given pressure resistance (Buchanan et al. 1971)
2. Cannot be operated remotely for extended periods of time — require operator attention.
3. Fluid losses into vacuum source can occur without careful monitoring (caused by reverse eddy).
4. Glass version is fragile for field use.
5. Peristalic pump not found reliable for field use (gravity feed is alternative but not easily controlled).
6. On recirculation, requires manual replacement of evaporated fluid (evaporation varies without ambient RH).
7. Requires careful cleaning but can become dirty during field operation is adequate fluid feed rate cannot be maintained.
8. Sample volume must be measured because fluid flow not constant.
9. Air flow should be regulated with critical orifice.
10. Washing organisms from walls not instantaneous may require additional scrubbing to recover total organisms. (Errington & Powell, 1969)
11. The "scrubbing" spray is effective in removing organisms after they were imparted onto the wall - not as aerosols. (Errington & Powell, 1969)
12. Vegetative bacteria (especially gram negative) damaged by cyclone as well as by other sampling devices (viability losses of 20-40% with E. coli)- using detergent-saline scrubbing fluid (mitigated by carbohydrate additions). (Erringotn & Powell, 1969).

TABLE 3, Cont.

Advantages	Disadvantages
	13. Although air flow rate quoted as 1000 1 pm, usually actually 650-850 1 pm.
	14. Liquid is supplied to "scent" spray about twice the rate of its subsequent recovery. Liquid not recovered is lost by creeping along walls of cyclone outlet and tube connecting it to vacuum source and by evaporation (F.P. Errington, private communication)
Viable Virtual Impactor	
1. Would permit particle size discrimination into two general classifications.	1. Will require fabrication.
2. May be used as a Class I or Class II sampler depending upon collection surface i.e. can deposit directly on assay surface or be washed for later assay.	2. Fabrication will be relatively expensive due to machining tolerances required for inertial impaction collection.
3. Robust design suitable for field studies.	3. Final collection surface requires additional evaluation.
4. Designed for long-term operation with minimal attention.	4. Requires balancing of airflow with critical orifices, which must be kept free of debris.
5. Depending upon the final collection surface used, particles from a very large air volume can be collected into a very small fluid volume.	5. Sampler efficiency and operational evaluation required.
6. Collection concepts have been shown effective.	
7. Collection surface can be changed depending on specific study objectives.	
8. Depending on collection surface used, recirculation of fluid with collected organisms not necessary.	

TABLE 4. RATIO OF POROUS WETTED SUBSTRATE TEST STAND TO SLIT SAMPLER FOR TOTAL AEROBIC BACTERIA-CONTAINING PARTICLE AEROSOLS[a]

Test Stand Fluid Flow Rate (ml/min)	Recovery Ratios								Geometric Mean
0.1	0.9	0.3	0.4	4.0	1.5	0.4	0.3	-	0.7
0.2	0.2	0.8	0.2	0.4	0.2	1.0	0.9	1.9	0.5
0.3	1.2	2.3	0.6	1.9	3.0	-	-	-	1.6

[a] Samples from empty animal holding rooms at 22° to 26°C, and 24% to 39% RH.

TABLE 5. RATIO OF POROUS WETTED SUBSTRATE TEST STAND TO SLIT SAMPLER FOR RECOVERY OF *B. SUBTILIS*- OR *S. MARCESCENS*-CONTAINING PARTICLES

Organism Aerosolized[a]	Recovery Ratios						Geometric Mean
BG	7.6	4.0	0.8	8.2	--	--	1.9
BG + SM	3.3	0.6	0.8	0.8	--	--	1.0
SM	1.8	0.2	1.0	1.2	1.1	0.2	0.7
SM + BG	0.3	0.2	0.2	0.1	--	--	0.2
Percent SM survival	9	38	20	18			19

[a] *B. subtilis* var. *niger* (BG); *S. marcescens* (SM).

the slit sampler. When aerosolized with *B. subtilis* var. *niger* spores, the geometric mean survival of *S. marcescens* after collection in the substrate test sampler was 19% of that observed in the slit sampler. Further observations using aerosols of *S. fecalis* , *E. coli*, and *E. aerogenes* showed that each organism was recoverable with both the slit sampler and wetted substrate test stand. As shown in Table 6, although *E. coli* and *E. aerogenes* were too numerous to count in aerosol samples collected with both samplers, the *S. fecalis* recovery ratio (3.05) showed substantially higher recoveries in the wetted substrate test stand than in the slit sampler. That is, assuming the probability of aerosol collection was similar to that observed in the two samplers when using *B. subtilis* var. *niger* aerosols, it appears that the *S. fecalis* survived well in the wetted substrate test stand.

These data suggest that the porous wetted surface is useful for recovering airborne particles containing bacteria. The lower recoveries of vegetative bacteria suggest that the particular test stand used may subject the collected vegetative microorganisms to lethal stresses. Such stresses may be reduced by test stand redesign, reducing exposure of collected organisms to the impact of the high velocity airstream, altering the composition of the collecting medium, and increasing the distilled water flow rate to replace evaporated fluid and prevent desiccation of the collected microorganisms.

Sampler Evaluation

Comparison of Glass and Stainless Steel Cyclone Scrubber Samplers

A glass and a stainless steel cyclone scrubber sampler (Figures 4 and 6) were compared to determine which should be used for fabrication of additional units. Preliminary studies using small particle sized aerosols ($\leq 1 \mu m$) of uranine dye-water and *B. subtilis* var. *niger* spore aerosols showed that the efficiency of aerosol recovery relative to the AGI-30 was very low with both samplers. Geometric average efficiencies, using both water and 25% trypticase soy broth (TSB) collecting fluids, were 14% in the glass and 5% in the stainless steel scrubber. When the uranine dye in the suspending fluid was replaced by *B. subtilis* var. *niger* spores, the geometric mean efficiency of the glass and stainless steel cyclone scrubbers for the recovery of these bacterial spore-containing aerosols was 9 and 12%, respectively.

To assess the sampling fluid losses within the two cyclone scrubber samplers, the collecting medium was seeded with uranine dye and non-dye-containing air was collected for 10 min periods. Prior to these trials, residual uranine dye in the test system was below the limits of detection. After collection, the fluid was adjusted to that of the original volume and the input and output dye concentrations were compared. As shown in Table 7, a greater percentage of the sampling fluid-contained dye was recovered from the glass than from the stainless steel scrubber. Successive washings showed about 11% of the total dye added could be recovered from the stainless steel scrubber while only 3% was recovered from the glass scrubber.

Since suspending medium viscosity can affect aerosol particle size, these observed low efficiencies might be partially attributable to inefficient sampling of very small sized aerosols generated from the low viscosity suspending

TABLE 6. RATIO OF POROUS WETTED SUBSTRATE TEST STAND TO SLIT SAMPLER FOR RECOVERY OF *S. FECALIS*, *E. COLI*, AND *E. AEROGENES* AEROSOLS

Organism	Recovery Ratios			Geometric Mean
S. fecalis	2.7	5.4	2.1	3.0
E. coli	T[a]	T	T	--
E. aerogenes	T	T	T	--

[a] T = collected bacteria formed colonies too numerous to count in both samplers.

TABLE 7. URANINE DYE RECOVERY FROM COLLECTING FLUID WITH STAINLESS STEEL AND GLASS CYCLONE SCRUBBERS

Sampler	Air Flow Rate (m^3/min)	Dye Recovered (%)	Water Rinse (ml)	Total Dye Recovered (%)
Glass	0.78	72	17	78
	0.75	72	32	74
	0.78	82	17	84
Geometric Mean =	0.77	75	21	79
Stainless Steel	0.90	56	22	62
	0.90	32	17	46
	0.90	68	32	82
Geometric Mean =	0.90	50	23	62

medium. To increase the size of the test aerosol particles to a range more commonly found in source-related microbial aerosols (e.g. > 2.1 μm, Carnow et al., 1979) and to enable a meaningful comparison of the two samplers, the viscosity of the spray suspension was increased by adding sucrose or dextran.

Employing the more viscous suspending medium for generation of *B. subtilis* var. *niger* aerosols improved the relative aerosol collection efficiencies of both scrubbers (Tables 8 and 9). Although wide ranges in collection efficiency were observed, the geometric mean relative viable aerosol collection efficiencies for aerosols ranging from 1.1 to 3.3 μm CMD were similar in both samplers: 52% in the glass and 56% in the stainless steel cyclone scrubber. The percentage of the original sampling fluid volume recovered in the glass cyclone scrubber was 30% while that of the stainless steel cyclone scrubber was 33%. No significant difference ($\rho = 0.05$) in either the relative bacterial spore or the sampling fluid recovery efficiencies was observed between the two samplers.

Because of the very similar performance of the glass and stainless steel cyclone scrubbers, selection for fabrication of additional units was based on the relative ease of fabrication. Although the glass scrubbers are more fragile and subject to breakage, they are less expensive and easier to fabricate. Thus, based on available evidence, additional glass scrubbers were fabricated for further study and for use by the U.S. EPA.

Relative Recovery Efficiencies of Glass Cyclone Scrubber for Aerosols Containing Different Organisms

Aerosols of *S. marcescens* and *B. subtilis* var. *niger* spores were collected with glass cyclone scrubbers. Table 10 shows the relative recovery efficiencies of the two microorganisms. The geometric mean relative efficiency for the recovery of *B. subtilis* var. *niger* spores was 68% and was 46% for recovery of *S. marcescens*. These significant differences ($p = 0.05$) in relative recovery efficiencies confirm that the survival of *S. marcescens* during the sample collection process was lower in the glass cyclone scrubber than in the AGI-30 sampler.

To determine the survival of *S. marcescens*, the relative concentration of *S. marcescens* and *B. subtilis* var. *niger* spores in the spray suspension was compared to that in the aerosol after collection with either the AGI-30 or the glass cyclone scrubber sampler. As shown in Table 11, the geometric mean survival of *S. marcescens* in the AGI-30 sampler was 40%, whereas the survival of the organism in the glass cyclone scrubber sampler was about 20% of that of the original suspension. While the survival of *S. marcescens* in the cyclone scrubber sampler was considerably lower than in the AGI-30 sampler, the volume of air sampled averaged more than 300 times greater in the former than in the latter device. The sampling sensitivity increased with the cyclone scrubber at the expense of viability.

Studies with f_2 coliphage-containing aerosols showed that the relative recovery efficiency of the glass cyclone scrubber compared to AGI-30 samplers was relatively high. The eight trials shown in Table 12 show that, although relative efficiencies were variable, the geometric mean aerosol recovery

TABLE 8. GLASS CYCLONE SCRUBBER RECOVERY OF B. SUBTILIS VAR. NIGER SPORE AEROSOLS[a]

AGI-30 Spores Recovered (cfu/m^3 X 10^5)	Sampling Rate (m^3/min)	Fluid Recovered (% of orig.vol)	Scrubber Spores Recovered (cfu/m^3 X 10^5)	Relative Efficiency (% of AGI - 30)
3.4	0.96	39	3.3	97
2.7	0.96	53	6.2	228
20.2	0.96	28	2.8	14
3.2	0.96	18	1.4	43
4.0	0.91	37	2.8	69
6.7	0.90	50	2.8	41
5.2	0.90	32	3.5	67
6.7	0.90	35	2.4	35
9.3	0.84	35	3.6	39
4.3	0.81	40	2.7	63
4.4	0.81	8	1.7	39
6.4	0.78	30	3.3	52
2.7	0.78	25	1.1	41
		$\bar{x}_G$ = 30		$\bar{x}_G$ = 52

[a] Aerosols generated from suspension containing 5% sucrose or dextran; particle size 1.1-3.3 μm CMD; temperature and RH not determined.

μm

TABLE 9. STAINLESS STEEL CYCLONE SCRUBBER RECOVERY OF B. SUBTILIS VAR. NIGER SPORE AEROSOLS[a]

AGI-30 Spores Recovered ($cfu/m^3 \times 10^5$)	Sampling Rate (m^3/min)	Fluid Recovered (% of original vol.)	Scrubber Spores Recovered ($cfu/m^3 \times 10^5$)	Efficiency (% of AGI-30)
4.6	0.96	31	2.6	56
4.6	0.96	27	2.6	56
15.6	0.96	17	0.2	3
5.2	0.93	42	14.0	270
2.4	0.93	31	1.5	63
4.6	0.92	49	7.0	153
5.8	0.90	40	5.3	91
24.1	0.78	42	9.8	41
		$\bar{x}_G = 33$		$\bar{x}_G = 56$

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[a] Aerosols generated from suspension containing 5% sucrose or dextran; particle size 1.1-3.3 μm CMD; temperature and RH not determined.

TABLE 10. GLASS CYCLONE SCRUBBER RECOVERY OF *B. SUBTILIS* VAR. *NIGER* SPORE- AND *S. MARCESCENS* AEROSOLS[a]

RH (%)	Sampling Rate (m^3/min)	Fluid Recovered (% of orig. vol)	*B. subtilis* var. *niger* Efficiency		*S. marcescens* Efficiency	
			($cfu/m^3 \times 10^4$)	(% of AGI-30)	($cfu/m^3 \times 10^4$)	(% of AGI-30)
33	0.88	22	8.6	56	6.7	24
28	0.82	28	9.6	36	158.5	29
53	0.81	50	11.2	69	27.5	45
31	0.75	20	8.4	52	43.3	42
79	0.78	25	b	--	14.9	31
79	0.78	27	b	--	15.4	35
56	0.78	20	11.8	91	11.9	c
64	0.74	53	b	--	23.2	44
91	0.74	55	16.2	65	36.1	38
83	0.74	53	b	--	58.3	76
63	0.74	52	17.3	36	13.5	104
38	0.69	20	15.7	97	4.5	36
57	0.68	47	b	--	17.6	28
57	0.66	55	12.7	99	0.8	b
57	0.64	10	20.9	76	13.1	170
				$\bar{x}_G = 68$		$\bar{x}_G = 46$

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[a] Aerosols generated from suspension containing 5% dextran; particle size 1.1-3.3 μm CMD; temperature 21°-26°C.

[b] AGI samples contained too few cfu for accurate enumeration.

[c] Contaminated AGI sample.

TABLE 11. *S. MARCESCENS* AEROSOL[a] SURVIVAL DURING AGI-30 AND GLASS CYCLONE SCRUBBER SAMPLING

RH (%)	*S. marcescens* Survival AGI-30 (% of Spray Suspension)	Scrubber (% of AGI)
28	100	7
38	40	38
44	33	76
39	13	154
57	27	33
57	27	200
57	108	b
64	72	b
56	25	40
	$\bar{x}_G = 40$	$\bar{x}_G = 50$

[a] Temperature 22°-26°C.

[b] Samples contained too few cfu for accurate enumeration.

TABLE 12. RECOVERY OF f_2 COLIPHAGE AEROSOLS[a] WITH GLASS CYCLONE SCRUBBER

RH, (%)	Sampling Rate, (m^3/min)	Fluid Recovered (% of original vol)	Efficiency (% of AGI-30)
28	0.82	28	46
79	0.78	27	102
56	0.78	20	33
51	0.76	37	123
83	0.74	53	106
b	0.74	28	58
54	0.74	37	335
51	0.62	15	29
			$\bar{x}_G$ = 76

---

[a] Aerosols generated from a suspension containing 5% dextran and *B. subtilis* var. *niger*. particle size 1.1-3.3 μm CMD; temperature 22°-25°C.

[b] RH data not determined.

efficiency of the scrubber relative to the AGI-30, was 76%, suggesting that the glass cyclone scrubber is a suitable device for recovery of virus-containing aerosols.

To determine the effectiveness of the glass cyclone scrubber sampler for detecting airborne animal virus, aerosols of poliovirus type 1 were sampled in four separate trials. Although virus concentrations in the suspending fluid were selected on the basis of preliminary f_2 coliphage studies to provide statistically reliable plaque counts in both the AGI-30 and glass cyclone scrubber samplers, the recovery was lower than expected in both samplers. Performance evaluations based on the data are shown in Table 13 and indicate that the glass cyclone scrubber was as efficient as the AGI-30 (92%) for detecting aerosols containing poliovirus.

The survival of f_2 coliphage was substantially higher than that of poliovirus type 1, which was contained in the same aerosol, in both the AGI-30 and glass cyclone scrubber samplers. Table 14 shows that, when using B. subtilis var. niger spores as the viability tracer, the geometric mean percent survival was 30% in the AGI-30 and 20% in the glass cyclone scrubber. That is, the f_2 coliphage survival in the scrubber was about 67% that observed in the impinger. Using the coliphage as a tracer organism, poliovirus survival was observed to be very low. As shown in Table 15, the geometric mean poliovirus survival was 1.00% and 0.26% in the AGI-30 and glass cyclone scrubbers, respectively. Under the conditions of this study, poliovirus was very fragile and died much more rapidly than coliphage in the aerosolization sampling process.

One possible explanation for the high poliovirus losses is that virus aggregation during sample collection might have caused an underestimation of the aerosol virus content. To consider this question, the samples collected with the glass cyclone scrubber were "deaggregated" and reassayed. After filtration through HIFCS pretreated 0.22 μm membrane filters, one aliquiot of each sample was filtered through a 0.05 μm polycarbonate filter. The 0.05μm filtered aliquot, together with a sample portion that had not undergone such filtration, was assayed in log dilutions on BGMK cell monolayers. The $TCID_{50}$ in these assayed portions of each poliovirus trial are presented in Table 16. No consistent differences were observed between the portions of the glass cyclone scrubber samples that were passed through 0.05 μm filters and those that were not passed through such filters. This indicated that the collected virus, of about 0.024 μm diameter (Melnick and Wenner, 1969), was not present in the sample in significant clumps. Thus, the low poliovirus survival, relative to f_2 coliphage, cannot be attributed to the assay but appears to represent a true loss of virus infectivity during the aerosolization and sampling process.

Discussion of Glass Cyclone Scrubber Sampler

The aerosol collection efficiency of the glass cyclone scrubber when compared to AGI-30 samplers varies substantially with the composition of the aerosol generating suspension and with the concentration of organisms being studied. The composition of the suspending medium may affect the aerosol particle size. Available data (Table 2) demonstrated that cyclone scrubber

TABLE 13. RECOVERY OF POLIOVIRUS TYPE 1 AEROSOLS[a] WITH GLASS CYCLONE SCRUBBER

Sampling Rate (m^3/min)	Fluid Recovered (% of original vol)	Aerosol Concentration (pfu/m^3 X 10^1)	Efficiency (% of AGI-30)
0.76	37	31.7	238
0.74	28	37.0	79
0.74	37	16.2	35
0.62	15	49.8	107
			$\bar{x}_G$ = 92

[a] Aerosols generated from a suspension containing 5% dextran, *B. subtilis* var. *niger* spores and f_2 coliphage; particle size 1.1-2.1 µm CMD; temperature 24°C; relative humidity 51-54%.

TABLE 14. SURVIVAL[a] OF f_2 COLIPHAGE IN AEROSOLS COLLECTED WITH ALL GLASS IMPINGER AND GLASS CYCLONE SCRUBBER SAMPLERS[b]

All-Glass Impinger	Glass Cyclone Scrubber
71	238
22	3
6	7
83	33
$\bar{x}_G = 30$	$\bar{x}_G = 20$

[a] Expressed as percent survival based on ratio of f_2 to B. subtilis var. niger in spray suspension and in aerosol sample.

[b] Temperature 24°C; relative humidity 51% to 54%.

TABLE 15. SURVIVAL[a] OF POLIOVIRUS TYPE 1 IN AEROSOLS[b] COLLECTED WITH ALL-GLASS IMPINGER AND GLASS CYCLONE SCRUBBER SAMPLERS

All-Glass Impinger	Glass Cyclone Scrubber
0.49	0.05
1.85	0.24
0.81	0.40
1.35	0.98
$\bar{x}_G$ = 1.00	$\bar{x}_G$ = 0.26

[a] Expressed as percent survival based on ratio of poliovirus type 1-to-f_2 coliphage in spray suspension and in aerosol sample.

[b] Temperature 24°C; relative humidity 51-54%.

TABLE 16. EFFECT OF PASSAGE OF GLASS CYCLONE SCRUBBER-COLLECTED POLIOVIRUS SAMPLE THROUGH 0.05 μm FILTER

Poliovirus TC1D$_{50}$ (−log 10 dilution)	
Unfiltered	Filtered
4.50	4.30
1.58	1.00
1.50	1.67
1.87	1.70

efficiencies were variable and dependent upon aerosol particle size, the specific test organism and collection fluid. Average efficiencies, using the AGI-30 as reference samplers, were 63% to 133% for B. subtilis var. niger, depending upon sampler configuration, particle size and collecting fluid. Using aerosols of about 1 to 3.5 µm CMD, substantial collection efficiency variability relative to the reference sampler was observed. The variability may be partially attributed to the great disparity in the volumes of air collected in the test and reference samplers. The air sampling duration with the AGI-30 was kept to 6 min to minimize excessive fluid losses. The air sampling duration with the cyclone scrubber samplers was 30 min. This sampling period was selected to reduce sampling error caused by the slower sampler response times and to more closely simulate sampling conditions required for detecting low concentration microbial aerosols. The data suggest that the very low ratio of AGI-to-cyclone scrubber air sampling rates, durations and resultant air volumes collected may cause substantial fluctuations in efficiencies due to the magnification of relatively small experimental error. For example, 0.075 m^3 AGI-30 samples (6 min at 0.0125 m^3/min) and 24 m^3 cyclone scrubber sample (30 min at 0.8 m^3/min) provide a sample volume ratio of 1:320. Expected experimental error due to sampling and plating can result in substantial fluctuation in apparent sampling efficiencies. There is a need for a viable reference sampler that is capable of sampling air volumes at the same order of magnitude as that of the device under evaluation.

Sampler sensitivity is affected by a number of factors and might be defined as:

$$CS = \frac{CE \times R \times D}{V}$$

where

CS — coefficient of sensitivity

CE — collection efficiency (% of reference sampler)

R — air sampling rate (m^3/min)

D — sampling duration capability (min)

V — final volume of collection fluid (ml)

Since the AGI-30 sampler was used as a reference device in this study, its aerosol collection efficiency (CE) is considered to be 100%. With an air sampling rate of 0.0125 m^3 per min for 6 min and a collection fluid volume of 20 ml, the coefficient of sensitivity could be calculated to be 0.38. Similar calculations of cyclone scrubber sampler sensitivity are presented in Table 17. The coefficient of sensitivity value in the cyclone scrubber samplers ranged from 17 with S. marcescens to 33 for poliovirus. Thus, under the condition of this study, the scrubber samplers were 45 to 87 times more sensitive than the AGI-30. Sampling sensitivity can, of course, be improved by increasing the air sampling rate aerosol collection efficiency and sampling duration or by decreasing the fluid volume required for the assays.

TABLE 17. EFFICIENCY AND SENSITIVY OF GLASS CYCLONE SCRUBBER SAMPLER WITH SEVERAL ORGANISMS

	Average Sampling Rate (m^3/min)	Efficiency (Geo. mean %)	CS
B. subtilis var. *niger* spores	0.88	52	23
B. subtilis var. *niger* spores aerosolized with *S. marcescens*	0.75	68	26
S. marcescens	0.75	46	17
f_2 coliphage	0.75	76	29
poliovirus type 1	0.72	92	33

One way to decrease this fluid volume is to recirculate the collection fluid during air sampling. Two basic problems, however, are associated with fluid recirculation. The first is the maintenance of fluid isotonicity, when a salt-containing medium is used, in the presence of evaporation. This could be maintained by adding water when the ionic strength of the fluid increases. Conductivity measurement with a relay suitable for solenoid activation could be used for liquid replacement. The second problem is the maintenance of viability of collected organisms during fluid recirculation, which depends to a large degree on the type of organisms collected. Fluid recirculation presents the risk of excessive stress and resulting viability losses.

The reliability of existing cyclone scrubber samplers is affected by the reliability of the fluid and air moving components employed in the system. The scrubbers utilizing a peristalic pump are subject to pump malfunctions, fluid flow variations caused by tubing variations with ambient temperature, and fluid losses due to excessive tubing wear. The use of reliable pumps that minimize fluid flow variations during the sampling process is recommended. Slippage of collection fluid from the sampler into the blower motor has been observed in the glass cyclone scrubber and is likely to occur unless the sampling process is closely monitored and adjustments in the fluid flow rate are made. Such fluid can result in blower malfunction or destruction during sampling. A fluid trap between the sampler and blower might reduce blower malfunctions but the air sampling rate may be reduced. Unless fluid losses are quantitatively accounted for, care should be exercised in the quantitative interpretation of collected data since true microbial aerosol concentrations are likely to be underestimated.

The cyclone scrubber samplers are easy to sterilize by autoclaving. Difficulties are encountered, however, when peristalic pumps are used since the tubing must be sterilized separately and aseptically connected to the sample and collection fluid reservoir. Use of a quick-connector device and a syringe-type pump would minimize both sampler set-up time and potential for presampling contamination.

Maintenance of collected sample viability requires an environment compatible with the survival of the specific organisms collected. Generally, this requires continuous hydration of the organisms in a collection medium at a temperature compatible with organism survival, but not growth. Evaluations of the cyclone scrubber (see Appendix) demonstrated the importance of collection fluid composition to the efficiency of microbial aerosol sampling. These evaluations do not, however, assess survival during long sampling periods or when collection fluid recirculation is necessary to achieve a required sensitivity level. Samples taken with the cyclone scrubber are collected into a fluid medium and are, consequently, suitable for assay by filtration and plating procedures for detection of various microorganisms.

The cyclone scrubbers are relatively easy to operate, compared to other existing large volume viable air samplers. The operation of this device requires some training and experience. The cyclone scrubbers cannot easily be remotely operated. Because of fluid evaporation fluctuations the fluid flow rates must be continually monitored in order to minimize fluid losses to the blower while maintaining a wetted cyclone surface. For field source monitoring, the cyclone scrubbers must be manually directed into the wind. Although cyclone scrubbers, in their present form, do not discriminate particle size, preimpactors or redesigned air sampler inlets could be used to accomplish such purposes.

The cost of cyclone scrubber fabrication is relatively low. The peristalic pump is the most expensive component of the cyclone scrubber, thus, necessity for frequent blower replacement can increase the costs of heavily used samplers. Glass blown cyclone scrubbers, which are relatively inexpensive to fabricate, are subject to greater sampler variations than are steel machined devices. Although under laboratory conditions a sampler may be highly efficient, the sampler inlet design and alignment, relative to wind direction will markedly affect the collection efficiency for various particle sizes under ambient environmental conditions. Since the ratio of wind speed to sampler inlet air speed cannot be held constant without elaborate instrumentation, a tolerable deviation from isokinetic sampling, and efficiency, must be accepted when wind velocity fluctuations occur.

APPENDIX

REVIEW OF MICROBIAL AEROSOL COLLECTION CONCEPTS AND APPLICATIONS

A. Sedimentation

Collection by sedimentation is based upon the settling property of aerosol particles onto a substrate. Widely used for recovery of both nonviable and viable aerosols, collection by sedimentation is affected by the terminal settling velocity of aerosol particles. This terminal settling was defined by Dimmick (1969) as

$$V_t = \frac{P_p D_p^2 g}{18\ \eta}$$

where:

V_t = terminal settling velocity (cm/sec)

P_p = density of particle (gm/cm^3)

g = acceleration due to gravity (cm/sec^2)

η = viscosity of air (gm/cm-sec)

D_p = particle diameter (cm)

Collection of aerosol particles by sedimentation is affected by both particle size and density. Large, dense particles are collected more efficiently than smaller and less dense particles.

Settling chambers and elutriators are examples of devices designed for nonviable aerosol collection by sedimentation. Settling chambers are opened in the test atmosphere and rapidly closed to entrap a representative air volume. The trapped particles are permitted to settle by gravity onto glass slides for microscopic examination (First, 1972). The horizontal elutriator, in its simplest form, is a rectangular channel shallow in depth compared to its length (Hemeon, et al., 1961). As an aerosol is passed slowly along a horizontal path, suspended particles settle to the channel bottom.

Airborne microorganisms have been collected by sedimentation onto various substrates. Puleo, et al. (1975), for example, described a method of collecting airborne bacteria for determination of the thermal resistance of spores by settling onto large surface area Teflon ribbons. Bacterial fallout has also been detected on stainless steel strips. The small collecting surface area of the strips used by Favero (1966), however, resulted in considerable variation between strips. One of the simplest and least expensive methods for microbial aerosol collection is the use of agar settling plates. The aerosol particles are permitted to settle on an agar-based medium in a petri dish. Following incubation, colonies are counted and the level of contamination for a defined time period is estimated. Settling plates were preferred by Gould (1970) over sieve samplers for use in hospitals because of convenience and quietness of operation. Ischenko, et al. (1962) preferred settling plates over an air centrifuge apparatus for collecting airborne bacteria. Although Fischer (1972) advocated sedimentation as a standard method for detection of bacteria in ambient air and Johnston, et al. (1978) reported higher recoveries of airborne bacteria with settling plates than with either Andersen or Nucleopore filter samplers, neither investigator considered the influence of desiccation on the viability of collected organisms during the long sampling periods. That is, differences in bacterial recoveries may reflect viability losses due to drying in the aerosol samplers.

Although settling plates determine the number of viable particles that adhere to the exposed plate, deposition on agar surface depends upon the air flow velocity and angle as well as particle size and density (Sykes, 1970). In studies comparing settling plates to slit samplers, for example, large particles were collected 200 times more effectively than smaller ones when using the sedimentation method (Bourdillon, et al. 1941). Deposition patterns may be influenced by Brownian diffusion with low settling velocities resulting in long collection times. Unit density particles of less than 0.5 μm diameter cannot be readily resolved by gravimetric sedimentation (Stober, 1964). Sedimentation methods are suitable only when the particles have a high terminal settling velocity, as with larger and denser particles. Thus, data obtained in this manner are biased in favor of larger particles. Since smaller particles are more likely to achieve deep lung penetration than are larger ones (Hatch, 1961), the value of settling plates in assessing the risk of respiratory infection is limited by their failure to detect the small aerosol particles.

B. Filtration

Filtration is widely used for collection of particle aerosols for measurements of mass, chemical composition, particle counting and sizing, and radioactivity. A filter consists of a porous material through which airborne particles are passed and separated from an air stream. Such filters can be made of fibrous mats of paper, glass, man-made fiber, woven industrial filter fabrics, granular beds, cotton, gelatin foam, and porous membranes. Air is pulled through the filters with a vacuum or, in some cases, a pressure pump. Highly porous filters allow penetration of some particles while more impervious filters offer great resistance to the air flow. Filter collection efficiency, however, decreases as the result of changes in the pressure drop across the filter, resulting from accumulated particle deposits.

Fiber filter efficiency is the result of particle capture by individual fibers and is given by

$$E_f = 1 - \frac{C_e}{C_o} = 1 - \exp - \frac{4L\ell\eta}{\pi D_f(1-\ell)}$$

where:

E_f = total filter efficiency

C_o = inlet concentration (®/cm^3)

C_e = exhaust concentration (®/cm^3)

L = filter total depth (cm)

D_f = filter fiber diameter (cm)

ℓ = filter packing density

η = individual fiber collection efficiency

The individual fiber collection efficiency, η, is determined by the particle size and fluid flow characteristics.

The most widespread applications of filtration procedures in aerobiology have been with cotton, gelatin, and membrane filters. A cotton filter consists of a wad of absorbent cotton packed into a small drying tube to a resistance of 38 cm H_2O at an airflow of 10 liter/min. Collected organisms are dislodged by shaking the cotton in an eluting medium such as water containing 0.1% Tween 80 (Wolf, et al., 1959). Cotton filters were found (Tyler, 1959) to remove more bacteria from the air than impinger samplers but, due to large viability losses of fragile microorganisms, this application enabled the recovery only of resistant forms. Cotton filtering devices were reported to retain all bacteria in aspirated air, with up to 98% of the collected bacteria recovered on three successive filter washes (Zubarev, 1960). Because of its high recovery efficiency of bacterial spores, the cotton filter was used as a reference sampler in evaluation of the recovery of *B. subtilis* var. *niger* spores with a simple liquid scrubber (Buchanan, et al., 1972) and has been used in microbiological safety surveys (Guyton, et al., 1956).

Gelatin filters can be solubilized after entrapment of microbial aerosols (Mitchell, et al., 1954), and bacterial yield from dissolved filters could be increased considerably by shaking with glass beads (Koller and Rotter, 1974). They reported high recoveries for 0.5 to 3.0 µm particles, with 25% higher counts than those on membrane filters. Noller and Spendlove (1956) reported that gelatin foam filters gave erratic results when compared to the all-glass impinger in field trials. The filters were also difficult to sterilize since treatment with ethylene oxide decreased their solubility. Gelatin filters were found by Haferkorn, et al. (1968) to be suitable samplers when high detection sensitivity and aerosol retaining ability were required but Rotter and Koller (1973) reported that increased sampling and filter storage times reduced recovery efficiencies. Automatic samplers employing gelatin filters have been used for detection of either the total number of bacteria or particles from air (Resch, et al., 1973; Rotter, et al., 1973). Commercially available water soluble filters were used by Rotter, et al. (1973) in an automatic sampler for up to 150 min sampling periods. Microorganism recovery was, however, influenced by the method of filter processing and was reduced by longer sampling times and high air sampling velocity rates.

Membrane filters have been widely used for diverse applications in microbiology. Although early studies showed significant variability in the membrane filter recovery of microorganisms (Wolochow, 1958), their use has been standardized for detection of microorganisms in water (Standard Methods, 14th ed. 1975). Some of the diverse applications of membrane filters, including their use in aerobiology, have been reviewed (Ehrlich, 1960; Mammarella, 1965; Spurny, 1967). Muir and Miline (1963) found that membrane filter samplers gave results comparable to a slit sampler for recovery of bacteria in aseptic rooms. Goetz (1953), however, showed that the aerosol recovery of unwashed vegetative S. marcescens was 0.1% to 2%. Jost and Fey (1970) demonstrated the applicability of membrane filter sampling for rapid bacterial aerosol detection using a fluorescent-antibody technique.

Air filtration samplers for detection of viable microorganisms are commercially available, simple to operate, and relatively inexpensive. As noted by Noller and Spendlove (1956) the use of filter samplers depends upon the ability of collected organsisms to resist drying on filters.

C. Impingement

Aerosol collection by impinging a high velocity airstream into a liquid medium is the principle employed by impinger samplers (Greenburg and Smith, 1922; Littlefield, et al. 1938). Such samplers are simple and easy to operate, with particle-containing air accelerated, following inlet tube entry, through a critical orifice before liquid impingement. The particles are retained in the collecting fluid while the airstream exits through the sampler outlet. These devices can be used for collecting both soluble and insoluble particles (Brown and Shrenk, 1938; Brown, et al. 1938; Littlefield, et al. 1938) and nonviable and viable aerosols.

Rettger (1910) described an air washer bubbler for collecting and enumerating airborne bacteria. This device was found by Bourdillon, et al. (1941) to be 60-90% as efficient as a slit sampler for collecting fine aerosols, but this efficiency decreased to 18% when sampling coarse aerosols. McConnell and Thomas (1952) used a thin layer of Japanese silk immersed in the collecting fluid of the "aeroscope". Although the wetted silk acted as a filter for removal of airborne bacteria, the collected organisms were difficult to cultivate. Apparent losses in viable microorganisms were due either to difficulty of removing the organisms from the silk or loss of viability in the aeroscope (Bourdillon, et al. 1941).

Armstrong (1970) described a device for sampling stack emissions for microorganisms. This sampler, which had a vacuum pump on one side of the base with a 700 ml wide-mouthed bottle containing 300 ml sampling fluid on the other side, was devised to cope with hot gases from stack emissions. The sampler was portable, mounted on a steel plate, and employed a stainless steel sampling probe for impingement of the sample into the collecting fluid.

The Greenburg-Smith impinger was first described as a dust cloud sampler (Greenburg and Smith, 1922) and was modified by Hatch, et al. (1932) for field sampling. This sampler has been used for studies in fields such as industrial hygiene and its application for collecting airborne bacteria was described by Wanner and Deuber (1969). The efficiency of liquid impingers for monitoring bacterial aerosols has been discussed by May and Harper (1957). The samplers are used to determine the total number of viable cells rather than the number of viable particles. Tyler and Shipe (1959) reported on the development and evaluation of the all-glass impinger (AGI) (Figure 1). Sprockhoff (1963) showed that a U.S.-designed liquid impinger device was superior to a German-built Drager pump membrane filter sampler. It was noted that collected organisms are more likely to survive collection in the liquid phase than dry filter collection.

The advantages and disadvantages of the AGI sampler were discussed by May (1966). The device is simple, inexpensive, easily sterilized and can, with a preimpinger, simulate the upper and lower respiratory system with respect to the aerosol particle size ranges collected.

The AGI was designed for isokinetic sampling of dynamic aerosols at $0.0125 m^3$/min. The sampler inlet tube was designed for retention of 50% of the 12 µm particles of unit density while excluding those particles greater than 18 µm (May and Harper, 1957). Although the AGI has been found to collect more than 99% of *B. subtilis* var. *niger* spores generated in 3 µm aerosols (Tyler and Shipe, 1959), Cown, et al. (1957) noted that re-atomization caused by striking an unyielding surface at sonic velocity can result in significant losses of collected organisms. In addition, considerable vegetative cell destruction in the sampler has been found to occur during high speed impingement (Tyler, et al. 1959). AGI samplers have been constructed with the jet-to-sampler-base distances at 4 mm and 30 mm, designated as the AGI-4 and AGI-30, respectively. The AGI-30 has been found to yield higher vegetative bacteria recoveries than the AGI-4 and has, subsequently, been suggested as a standard sampler (Brachman, et al. 1964).

To minimize viability losses due to direct impingement, tangential impingement has been used. Tyler, et al. (1959) described a tangential impingement sampler with subsequent splashing and washing, to collect airborne microorganisms at 9.5 liter/min into 10 ml collecting fluid. The bulged AGI, another tangential impinger, sampled 12.5 liter/min of air into 10 ml collecting fluid. It was designed to minimize the impingement shock rendered to aerosolized microoganisms when collected by such samplers. In the Shipe impinger, air strikes the sampler base tangentially, causing a nonsplashing fluid rotation (May and Harper, 1967). This sampler was designed to avoid sonic impingement and to prevent possible size discrimination in the collection of viable aerosols larger than 3.0 µm MMD (Shipe, et al. 1959). In comparative studies, the Shipe sampler gave *S. marcescens* recoveries that were as good as or better than those of either the AGI or Venturi scrubber for particles of 3 µm MMD (May and Druett, 1957). Tyler, et al. (1959) reported that the viability losses of vegetative bacteria were smaller in the Shipe sampler than in the AGI because of the angle of impingement. Couch, et al. (1965) adapted a modified Shipe impinger for sampling coxsackievirus A21 aerosols. Of the total atomized coxsackievirus A21, 50% was recovered with a Shipe impinger by Gerone, et al. (1966).

The midget impinger is a low speed bubbling and washing device that samples at subsonic velocities into 10 ml collecting fluid at a sampling rate of 2.8 liter/min. The sampler was as effective as the AGI for small droplets and more effective for larger ones (Tyler and Shipe, 1959). The capillary impinger was described as sampling at 1.9 liter/min into 25 ml fluid at sonic velocity (Tyler and Shipe, 1959) and was used at a maximum flow rate of 2.5 liter/min (Harstad, 1965).

A fritted bubbler, for air washing into about 15 ml of liquid medium, was designed for subsonic sampling at 1.0 liter/min (Tyler and Shipe, 1959). The sampler had a fritted glass disc at the end of the air inlet tube for intimate gas-liquid washing by the production of uniform distribution of bubbles. The samplers were less efficient than the AGI for recovery of B. subtilis var. niger and S. marcescens contained in small particles.

Air sampling directly onto cell cultures was reported by Hankins and Hearn (1970) to be more sensitive than the AGI for Venezuelan equine encephalomyelitis virus aerosols. It is likely, however, that such a method requires a relatively concentrated viral aerosol since exposure to large volumes of air is likely to be injurious to the cell culture system.

Noble (1967) indicated that the most satisfactory recovery of virus from aerosols appeared to involve a fluid sampling medium, such as that used in liquid impingers, followed by the medium's inoculation into a susceptible host. The efficiency of the AGI was found to increase significantly for T-3 phage, but not for mengovirus-37A or vesicular stomatitis virus recovery when the air was humidified prior to sampling (Warren, et al. 1969). Noting that higher T-3 coliphage and Pasteurella pestis (sic) phage recoveries occurred with inlet air prehumidification, Hatch and Warren (1969) suggested that the AGI alone could underestimate virus aerosol concentrations by up to 3 logs. Impinger slippage was reported by Harstad (1965) to vary inversely with relative humidity, probably due to particle size changes.

While the AGI has been recommended for use as a standard sampler (Brachman, et al. 1964), this device is at a severe disadvantage in the sampling of aerosols containing low concentrations of microorganisms often encountered in field studies. Because of its relatively low sampling rate, cooling and evaporation may occur when long sampling times are required (Anderson and Cox, 1967). Since the liquid is under low pressure, it evaporates quickly and tends to freeze in cool, dry air. Changes in sampling fluid pH, volume, and osmotic characteristics can also occur with extended sampling times (Hyslop, 1972). Depending upon the sampling fluid used, an antifoaming agent may be required to avoid excessive foaming.

To increase the sampling capacity of the AGI, operation of several impingers in parallel, with their collection fluid pooled for assay (Hyslop, 1965), or the use of multiple jet impingers (Thorne and Burrows, 1960) has been proposed. The multiple jet samplers were efficient for foot-and-mouth disease virus detection and detection sensitivity was enhanced by the application of an adsorption and elution technique. A continuous impinger was reported by Goldberg and Watkins (1965) to permit sampling at higher flow rates (0.0283 m^3/min) and for longer time periods than the AGI. Sampling fluid was continually added and removed from the device by a peristalic pump. The continuous impinger, however, was found to be only 47% and 61% as efficient as the AGI-30 for recovery of *E. coli* and *B. subtilis* var. *niger* spores, respectively.

Larger capacity samplers have been developed in an effort to collect aerosols from relatively large volumes of air into a fluid medium. An inertial sampler was designed to collect particles by inertial impaction from 1000 liter/min of air onto liquid-coated wire spokes attached to a rapidly spinning disc (Decker, et al. 1969). This sampler was not successful due to several problems including failure of the liquid to remain on the leading edge of the spinning spokes where the particles impacted and the splashing of collection fluid at the rim of the spinning disc. The slit liquid impinger collectors (Decker, et al. 1969) were developed to collect aerosols at a rate of 200 liter/min of air with 50% collection efficiency for 2 μm particles. The particles were collected by inertial impingement into a liquid film on a rotating disc. Problems were encountered, however, during aerosol collection trials due to uneven liquid film distribution.

The multislit impinger (MSI) (Peterson, 1971) operates by inertial impaction of airborne particles onto a liquid film spread over a rotating disc by centrifugal force and a wiper arm. Particle-laden fluid is removed from the disc with a pickup arm and pumped by a peristaltic pump to a storage or dumping container. Impaction is achieved by accelerating the particles within an airstream passing through six slits positioned radially above the collection disc. With collection efficiency calculations based upon an assumed particle density of 1.4 g/cm^3, sufficient inertia was attained for collection, with 50% efficiency, of the particles of 1.0 μm. Buchanan, et al. (1968) showed that the MSI had an averge collection efficiency of 82% for *S. marcescens* and 78% for *B. subtilis* var. *niger* spores when compared to the AGI-30.

The applicability of the MSI for extensive field microbiological aerosol monitoring is limited. For field studies the MSI must be more reliable, portable, require less operator attention, and be capable of more remote operation, utilize a fluid-moving method that would maintain a uniform flow rate, and reliably maintain a completely wetted disc. The "reliability, maintainability, and portability was considered to be of less importance" in the MSI design than demonstrating the stated theory of operation (Peterson, 1971). Problems have been caused by the liquid distribution and pickup, placement and location of nozzles, disc drive assembly, and liquid flow and air flow devices. Fannin, et al. (1976) found the MSI to be undependable and subject to frequent breakdowns in field operations. In addition, the sampler design does not lend itself to conventional sterilization procedures.

Attempts to assess the respiratory hazards of microbial aerosols have motivated development of aerosol size discriminating modifications to impingers. A preimpinger has been used, for example, at the AGI air inlet to collect non-respirable particles while permitting those in the respirable size range to be collected by the sampler (May and Druett, 1953). May (1966) described a multistage liquid impinger to determine the numbers of microorgamisms associated with given particle size ranges. Depending upon the size of the unit, a sampling rate of up to 50 liter/min was obtained. The sampler, made of Pyrex glass, was designed to minimize evaporation and permit prolonged sampling periods. Particles were collected with a subcritical jet, resulting in more gentle impingement and less pressure drop than in the AGI. For sampling into the wind, a hemicyclindrical metal shield sampler inlet attachment was described. In a 10 mph wind, the sampler intake efficiency for 15 μm particles was found to be 9.6% without the intake baffle and 99% with it. "Stagnation point," rather than isokinetic, sampling resulted with use of the fitted shield which was essential for efficient recovery of large particles under windy conditions. May (1966) emphasized that isokinetic sampling cannot be achieved from a horizontally moving air stream involving impaction onto a horizontal liquid surface, and a 90° bend. Because of air turbulence, true isokinetic sampling can be achieved only inside a laminar flow wind tunnel.

D. Precipitation

a. Electrostatic

Electrostatic precipitation takes place when a force is exerted on a charged particle by an electric field. Many aerosol particles carry weak charges, but for improved collection, additional charging is required (Machala, 1965). An electrostatic sampler is a device that removes fine suspended particles from a gas by charging the particles by either a corona discharge or irradiation from a radioactive source (Kraemer and Johnstone, 1955; Liu, et al. 1967; Liu and Verma, 1968).

A fundamental quantity characterizing the performance of an electrostatic sampler is the particle migration velocity (White, 1963), i.e. the velocity exhibited by a charged particle moving toward a collecting surface under influence of an external electric field. The precipitating force F_s is (Dennis, 1976):

$$F_s = E_p \cdot Q$$

where

E_p = precipitating electric field intensity

Q = particle charge

Assuming Stoke's law is valid, the particle migration velocity, W, is

$$W = \frac{F_s C}{6\pi\eta a}$$

where

η = viscosity of gas

a = particle radius

C = Cunningham slip correction

Particle motility, B, can then be written as (Kuehne, 1961)

$$B = \frac{W}{E_p} \, \frac{QC}{6\pi\eta a}$$

The sampler collection efficiency, CE, is found by taking a mass balance of material lost from the stream and material deposited on the plane.

$$CE = 1 - \frac{\exp(A\ B\ E_p)}{V_g}$$

where

A = total surface area of collection

B = particle mobility

E_p = intensity of electric field

V_g = volume flow rate of gas

Lower flow rate type samplers have a higher collection efficiency than with higher flow, and may be used for aerosol size measurements. Electrostatic precipitation samplers are most suitable for sampling particles with diameters ranging from about .05 to 5 µm at flow rates from 2 to 5 liters/min (Dennis, 1976).

The concept of electrostatic precipitation for collection of bacterial aerosols was described by Berry (1944). Luckiesh, et al. (1946) described an electrostatic precipitator containing two agar plates charged with 7-10 KV. As air was drawn over the surface, particles carrying a charge were deposited onto the oppositely charged agar surface. Morris, et al. (1961) found that high recovery efficiencies were attainable by electrostatic precipitation with aerosols of four different species of bacteria and a bacteriophage.

A tubular electrostatic precipitator was described by Houwink and Rolvink (1957) for the assay of bacterial aerosols. This device consisted of a high tension electrode centrally mounted inside an agar-or fluid-lined cylinder. The device was, however, not suitable for rapid sampling of consecutive aerosols, small air volumes, high bacterial concentrations, and aerosols of wide concentration ranges. To improve the sampler, three tubular electrostatic precipitators were described (Morris, et al. 1961). One sampler contained an agar or agar-impregnated collector, the second was modified for small air volume collection, and the third contained a fluid collector. The collection efficiency of the electrostatic precipitator was high, although it was affected by voltage variations. Comparisons of the fluid sampler with the all-glass impinger for recovery of T-3 phage in 90% RH showed poorer recoveries of viable phage for the former device. These low recoveries were attributed to the effects of ozone generated in the electrostatic precipitator on phage viability. Although alteration of the collecting fluid can afford some protection, the potential effects of ozone on the viability of the collected organism should be considered in sampler designs.

The Porous Electrode Electrostatic Precipitator (PEEP) (Decker, et al., 1969) sampled at 100 liter/min pulling air through a corona generated by a ring of negatively charged needles. Negatively charged aerosol particles were precipitated onto a porous electrode wetted by pumping a conductive collection fluid through a frit. As particle-laden collection fluid spilled over the outside of the porous electrode, a liquid level sensor was used to drive a makeup pump for replacing the continuously collected conductive fluid. The PEEP sampler had a collection efficiency of 20% for 0.9 µm *B. subtilis* var. *niger* spores and 1.3 µm latex spheres, with particle holdup on the porous electrode constituting a major design problem.

The Rotating Electrode Electrostatic Precipitator (REEP) sampler had a rotating anode continuously wetted by a conductive collection fluid maintained in a liquid film over the disc surface by a wiper (Decker, et al. 1969). The air entered at 100 liter/min through a corona generated by negatively charged needles with charged aerosol particles collected onto the positively charged liquid film. The particle-laden fluid was recovered via a pump for subsequent assay. The collection efficiency of the REEP sampler for 0.8 to 1.0 µm *B. subtilis* var. *niger* spores and 1.3 µm polystyrene latex spheres was 50%.

The Space Charge Aerosol Precipitator (SCAP) was designed to collect aerosols at 100 liter/min onto charged collection fluid droplets. The droplets and charged aerosol particles then moved to a grounded fluid that drained down a cylindrical wall into a collection trough. The collection efficiency for *B. subtilis* var *niger* spores (0.9 µm) was 35% with apparent losses on the cylinder walls (Decker, et al. 1969). In the Environmental Research Corporation Electrostatic Coagulation Sampler, charged particles were removed with a spray of oppositely charged collection fluid droplets from 1000 liter/min of air (Decker, 1969). The particle-laden droplets were then forced to the cylinder walls by a high voltage central rod. The efficiency of this device was 15% with *B. subtilis* var *niger* spores.

Electrostatic precipitation has been found to be less effective than liquid impingement for detecting some viable airborne viruses (Hyslop, 1970). Hyslop (1971) suggested that the viability impairment could be the result of rapid evaporation of polar liquids enhanced by an electric current across liquid surfaces or because an atmosphere containing more than a critical concentration of small unipolar air ions increases evaporation relative to the nonionized atmosphere. Although physical changes in the aerosol during electrostatic precipitation are not completely understood (Agafonova and Matulyavichus, 1968), such aerosol collection concepts have been found effective for collection of certain microorganisms, including phages (Grigoreva, 1965; Grigoreva, 1963).

Electrostatic precipitation concepts were employed in the development of air samplers for detection of airborne microorganisms from relatively large volumes of air. Much of the initial work on large-volume air sampling was performed under contract with the U.S. Army by Litton Systems, Inc. and by the Environmental Research Corporation (Decker, et al. 1969). In an effort to design a more sensitive methodology, more complex instrumentation was developed. The Litton Systems large volume air sampler (Figure 11) employed the electrostatic precipitation of airborne particles onto a continuously wetted rotating disc as air was pulled through the instrument. The development of the LEAP sampler, operating on similar principles, was described by Lundgren and Graf (1964).

Such devices, designed for operation at an air flow rate of either 1 or 10 m^3/min, have been used for detection of relatively low concentrations of airborne bacteria and viruses. Artenstein, et al. (1967), for example, documented the feasibility of indoor sampling with the LVAS for recovery of adenovirus aerosols in a military recruit camp during an acute respiratory disease epidemic. Previous attempts to detect airborne viruses with lower volume samplers were less successful (Artenstein, 1964). Winkler (1968) isolated rabies virus with LVAS samplers after attempts with all-glass impingers were unsuccessful. Likewise, murine leukemia virus has been recovered using a LVAS when the agent could not be detected with an AGI (Larson, et al. 1970). The recovery of airborne foot-and-mouth disease virus has been studied with LVAS sampler (Donaldson, et al. 1970; Sellers and Parker, 1969). Hugh-Jones, et al. (1973) provided evidence for the airborne spread of Newcastle disease virus with a LVAS.

A comparative evaluation of the multi-slit impinger (MSI) and the LVAS showed that their efficiency rates compared to the AGI-30, varied with the organisms assayed. For S. marcescens, the LVAS had a 95% efficiency, compared to 90% for the MSI. With B. subtilis var. niger, the LVAS showed a 74% while the MSI a 86% efficiency (Decker, et al. 1969). Problems with evaporation and quantitation with large-volume samplers have been discussed by Gerone, et al. (1966).

The LVAS has been found effective for aerosols containing low concentrations of bacteria, virus, and rickettsia. The sampler was most effective in still air when operated in a level position, constantly monitored by a trained technician, and not subjected to electric current fluctuations (Curtis, 1969). If collecting fluid evaporation increases the relative humidity within the sampler, increased die-off of airborne microorganisms is likely to occur since ozone is most effective as a germicide at high relative humidity and low temperature (Gilgren and Wanner, 1966).

b. Thermal

Aerosol particles in a thermal gradient, i.e., between a heated and a cooled surface, tend to move in the direction of the lower temperature (Cawood, 1936; Watson, 1936). If a microscope slide is placed on the lower temperature surface the thermal force causes the particles to precipitate on the slide. Complete, or nearly complete, collection is usually achieved with a thermal precipitator for particles greater than 0.05 μm diameter. Thermal precipitators have sampling rates of from 7 cm^3/min to about 0.5 liter/min. A high temperature gradient between hot and cold surface is needed for complete

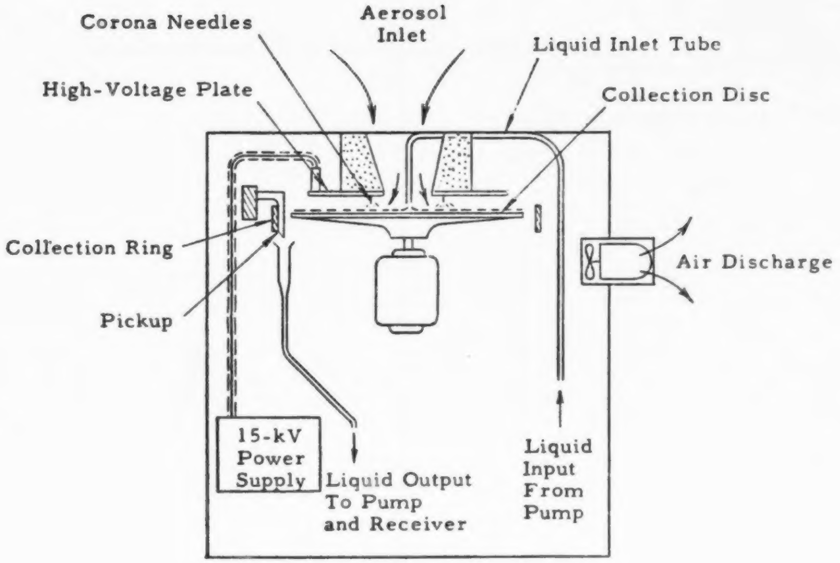
Corona Needles
Aerosol Inlet
Liquid Inlet Tube
High-Voltage Plate
Collection Disc
Collection Ring
Air Discharge
Pickup
15-kV Power Supply
Liquid Output To Pump and Receiver
Liquid Input From Pump

FIGURE 11. Large volume electrostatic precipitator air sampler

collection of aerosols. This is usually achieved by making the distance between the hot and cold surface small (Kethley, et al. 1952).

A thermal precipitator described by Kethley, et al. (1952) was used by Orr, et al. (1956) to collect airborne microorganisms onto nutrient broth-saturated filter paper. An air sampling rate of 0.3 liter/min was used and microorganism recovery was compared to midget and critical orifice impingers. The recovery of bacterial aerosols with the thermal precipitator was at least as efficient as with the other two samplers. Thermal precipitation is attractive for aerosol sampling because of the high efficiency of collection. Disadvantages of thermal precipitation are the long time required to obtain the sample when aerosol concentration is low and non-random deposition with respect to size.

E. Centrifugal Separation

Particles suspended in a gas stream moving in a circular path tend to move outward radially. Centrifugal collectors differ from gravity collectors in that the force of gravity is replaced by force equal to

$$F_c = \frac{mv^2}{R}$$

where

F_c = centrifugal force

m = mass of particle

v = tangential velocity of the gas stream

R = radius of curvature

The drift velocity due to the above centrifugal force is

$$V_d = \frac{T.V^2}{R}$$

where

V_d = drift velocity due to centrifugation

T = relaxation time of particle

V = velocity of gas stream (tangential)

The two basic types of centrifugal devices, namely, centrifugation and cyclone separators, differ in their methods of generating the centrifugal force.

a. Centrifuges

The centrifugal force in air centrifuges is generated by rotating the body of the devices. The air sampling rate depends upon the characteristics of the specific centrifuge. In a conical centrifuge, airborne particles flow through an annular between two concentric cones (Sawyer and Walton, 1950) while in a spiral centrifuge the aerosol flows through a rectangular spiral channel. As the aerosol moves from the inlet to the outlet of the device, centrifugal force is increased due to the increase in the curvature radius. Air centrifuges can be designed to collect particles greater than 0.1 µm in diameter, with the larger particles collected first (Kast, 1962; Goetz and Preining, 1960).

Wells (1933) described an air centrifuge for detecting airborne bacteria in which melted agar medium was placed in the centrifuge cup for sample collection. The sampler was not suitable for areas of high bacterial density (Wells, 1933) and was inadequate for collection of small droplet nuclei (Phelps and Buchbinder, 1941). The efficiency of the Wells centrifuge increased with larger particles. Considerable care was required in preparation and counting of its culture tubes (Bourdillon, et al. 1941). Another air centrifuge was described by Rechmenskiy (1957) in which a plastic rotating disc was connected to a fan motor shaft. As the disc rotated, bacterial aerosols were impacted onto the surface of cellophane sheets after being drawn through inlet tubes.

A centrifugal air samples, introduced by Folex-Biotest-Schleussner, Inc. (1979), was designed to impact particles onto nutritive agar-containing plastic strips at an air sampling rate of 40 ℓpm. This sampler was descibed as a hand held battery powered device that offered the advantage of complete portability for detecting microbial aerosol.

b. Cyclones

Rather than rotating the body of the device, cyclones are designed so that the fluid is rotated resulting in centrifugal forces. The collection efficiency of cyclones decreases rapidly with particles less than 5 µm in diameter (Brookman, et al. 1963). Cyclone separators are particularly useful for particle collection from gas streams at high temperatures and pressures (Orr, 1966) and for separation of particles into respirable and nonrespirable fractions. Four different cyclone scrubber samplers have been described for use as ambient viable microbial air samplers. A general description of each sampler is shown in Table 18 and the performance data are presented in Table 19. All four cyclone scrubber samplers appear to collect impacted aerosol particles by fluid washing with a fine liquid spray.

Errington and Powell (1969) designed a cyclone separator for ambient viable microbial air sampling. Two models with 0.075 m^3/min and 0.35 m^3/min sampling rates were constructed of either Perspex® or stainless steel. The device was intended to collect 50% of 0.85 µm and 100% of 2.2 µm particles, based upon data and discussions of Fuchs (1964), Davies (1952) and Lapple (1950). The smaller device concentrated 0.1 m^3 of air into 1 ml of collecting fluid. The collecting fluid was spray injected at 0.5 to 2 ml/min and thrown

Table 18. CYCLONE SCRUBBER DESCRIPTIONS

Sampler	General Description	Reference
Porton Cyclone Separator	Made in two sizes (71 l/min and 350 l/min) in either Perspex or Stainless steel. In the smaller version, 1 in^2 throat gave average acceleration of 10^7 cm/sec^2 at top of cyclone. Main body constructed in two parts. Fittings are machined. Inlet tube between annular gap between two plates to exclude large particle. The outer plate carries a fine tube at its center for scrubbing liquid feed. Samples collected in bottles screwed into hood enclosing tail pipe. Scrubbing liquid supplied by peristaltic pump giving a continuous flow at 0.5 to 2 ml/min. Fluid loss due to reverse eddy and evaporation. Airflow regulated by critical orifice between cyclone and pump.	Errington and Powell (1969)
Aerojet-General Scrubber	Sampler consists of a spray device, a glass coalescence tube, and a cyclone mist separator containing a wash tube.	Decker et al (1969)
Simple Liquid	Collecting fluid pumped through needle into throat of inlet arm 1.9 cm diameter where it is aspirated into a fine mist by the airstream. Air enters right angle arm tangentially and assumes a spiral path. The mist droplets are thrown out of the airstream onto the walls, forming a liquid film moved helically by the air-stream to an outlet where the fluid is collected in a flask with a slight vacuum. Airborne particles primarily removed by impaction onto liquid film. Prototype made of acrylic resin.	Buchanan et al (1972)
DRES-modified cyclone scrubber	Similar to the "simple liquid scrubber" with the following differences: (1) the portion of the transition piece that is inserted into the collector is redesigned to collect all fluid; (2) vacuum for fluid collection is provided by a pressure drop between sample outlet tube and motor (rather than by a peristaltic pump); (3) collecting fluid is provided at a constant rate by a screw-driven syringe (rather than by a peristaltic pump); (4) water lost by evaporation is added by a special metering device; (5) particles ≥ 10 μm MMD can be excluded by a removable preimpactor.	White et al (1975)

TABLE 19, SUMMARY OF CYCLONE SCRUBBER EVALUATIONS

Sampler	Reference	Collection Fluid	Aerosol Particle Size (um)	Test Organism	Sampling Rate (m^3/min)	Duration (min)	Fluid Vol. (ml)	Reference Sampler	Efficiency (% of Reference Sampler) Mean	Range
Cyclone Separator	Errington and Powell (1969)	Saline	N.A.[a,b]	BG	0.075	N.A.	2	AGI-30	80[c]	N.A.
		Saline	N.A.[b]	BG	0.075	1	N.A.	AGI-30	76[c]	N.A.
		Special[d]	N.A.[b]	BG	0.075	1	N.A.	AGI-30	85	73-97
Aerojet-General Scrubber	Decker et al. (1969)	HBSS+ LAH	N.A.[e]	BG	1.000	5	N.A.	AGI-30	63	N.A.
		HBSS + LAH	N.A.[e]	SM	1.000	5	N.A.	AGI-30	70	N.A.
Simple Liquid Scrubber	Buchanan et al. (1972)	0.1% Tween 80	0.9	BG	0.850	10	20-40	Cotton	71	65-77
		0.1% Tween 80	1.8	BG	0.850	10	20-40	Cotton	90	85-95
		0.1% Tween 80	3.6	BG	0.850	10	20-40	Cotton	96	90-102
		HBSS + 0.5%LAH	0.9	BG	0.950	5	11-14	AGI-30	44	36-56
		HBSS + 0.5%LAH	1.8	BG	0.950	5	11-14	AGI-30	90	90-109
		HBSS + 0.5%LAH	4.0	BG	0.950	5	11-14	AGI-30	103	87-128
		0.6% Tween 80	0.9	BG	0.950	5	11-14	AGI-30	95	84-104
		0.6% Tween 80	1.8	BG	0.950	5	11-14	AGI-30	133	110-156
		0.6% Tween 80	4.0	BG	0.950	5	11-14	AGI-30	110	92-137
DRES-Modified Cyclone Scrubber	White et al. (1975)	0.6% Tween 80	N.A.[f]	BG	0.950	3	3-6	AGI-30	82	62-102
		0.6% Tween 80	N.A.[f]	SM	0.950	3	3-6	AGI-30	K.A.[g]	K.A.[g]
		0.6% Tween 80	N.A.[f]	AA	0.950	3	3-6	AGI-30	70	57-83
		0.6% Tween 80	N.A.[f]	EC	0.950	3	3-6	AGI-30	K.A.[g]	K.A.[g]
		0.5 G + M[h]	N.A.[f]	BG	0.950	3	3-6	AGI-30	82	66-98
		0.5 G + M	N.A.[f]	SM	0.950	3	3-6	AGI-30	89	69-109
		0.5 G + M	N.A.[f]	AA	0.950	3	3-6	AGI-30	87	56-119
		0.5 G + M	N.A.[f]	EC	0.950	3	3-6	AGI-30	79	74-83
		GGMP[i]	N.A.[f]	BG	0.950	3	3-6	AGI-30	86	62-110
		GGMP	N.A.[f]	SM	0.950	3	3-6	AGI-30	70	55-85
		GGMP	N.A.[f]	AA	0.950	3	3-6	AGI-30	33(K.S.)[j]	25-41 (K.S.)[j]
		GGMP	N.A.[f]	EC	0.950	3	3-6	AGI-30	45(K.S.)[j]	37-63 (K.S.)[j]

TABLE 19, Cont.

Sampler	Reference	Collection Fluid	Aerosol Particle Size (um)	Test Organism	Sampling		Fluid Vol.	Reference Sampler	Efficiency (% of Reference Sampler)	
					Rate (m^3/ min)	Duration (min)	(ml)		Mean	Range
DRES-Modified Cyclone Scrubber	Park et al. (1978)	MEM+ B+ A[k]	N.A.[b]	CV	0.002[1]	N.A.	N.A.	AGI-30	39	32-43
		MEM+ B+ A[k]	N.A.[b]	RV	0.002[1]	N.A.	N.A.	AGI-30	58	46-67
		MEM+ B+ A[k]	N.A.[b]	PV	0.002[1]	N.A.	N.A..	AGI-30	38	15-59[m]
Glass Cyclone (UK)	Lejuene (Personal Communication)	N.A.	N.A.	BG	("slightly less efficient than DRES-Modified Cyclone Scrubber")					
		("recovery improved with BSS or nutrient broth)	N.A.	T-1	("slightly more efficient than DRES-Modified Cyclone Scrubber")					
			N.A.	MS-2	("slightly more efficient than DRES-Modified Cyclone Scrubber")					

[a] N.A.: information not available.

[b] Collison nebulizer used. Reported to produce aerosols in which 90% particles <3μm MMD.

[c] Efficiency increased with subsequent cyclone washing.

[d] Collection fluid consisted of sodium chloride, 0.11M, sodium phosphate (pH 7.8), 0.02M, together with 0.1% w/v of lauryl ether of polyethylene.

[e] Vaponefrin nebulizer used. Reported to produce aerosol particle sizes of approximately 2μm MMD (Larson et al., 1976).

[f] Collison (90% of particles <3μm MMD) or Venturi type needle (70% of particles ≤3μm MMD) nebulizer used.

[g] No results: organism killed in AGI-30.

[h] 0.5 gelatin + milk.

[i] Glycerol gelatin milk phosphate.

[j] K.S.: organism killed in scrubber.

[k] MEM + 0.1% BSA, Antifoam C.

[l] Flowrate reported to be 1.6 lpm.

[m] Lowest efficiency with lowest concentrations.

to the cylinder walls causing an uneven wall covering film as it moved helically toward the lower apex and was drawn by slight suction into a receiving container. The scrubbing liquid was supplied through a peristalic pump. To account for evaporation losses, the fluid flow rate required manual adjustment. Washing of organisms from the collected wall was not instantaneous, however, and several post-collection washings were required to maximize the recovery. Washing efficiency was influenced by collecting fluid composition and increased with the addition of non-ionic detergent. As compared to the AGI, the cyclone separator recovered 85% B. subtilis var. niger spore aerosols. E. coli viability losses ranged from 20% to 40% but actual viability losses depended upon the sampling fluid composition.

A sampler designed for scrubbing up to 20 m^3 of air by microfog was described by Fontanges and Isoard (1973). A cyclone separator was used for removal of 10 µm sized particles prior to scrubbing. An aspirating tube with a Venturi form caused rapid air expansion which resulted in instantaneous condensation on microparticles which become droplet nuclei. Convergence followed by divergence decelerated the microfog which was collected with a cyclone. Although particles 1 µm were reportedly retained, the apparatus was rooftop mounted, lacking both the portability and robustness required for field studies.

Using the concept of the Cyclone-Scrubber (Decker, et al. 1969), Buchanan, et al. (1972) described an easily fabricated "simple liquid scrubber". This device concentrated airborne microorganisms into a small liquid volume at a ratio of 400,000 to 1, at an air flow rate of 0.85 to 0.95 m^3/min and a fluid flow rate of 2 to 4 ml/min. The collecting fluid was pumped with a peristaltic pump through a needle into the throat of an inlet arm and aspirated into a fine mist. The mist droplets were thrown out of the airstream by spirally moving air, which tangentially entered the right angle arm, forming a continuous fluid film in which airborne particles were impinged. The particle-laden fluid was then collected in a flask. The collection efficiency of B. subtilis var. niger spore aerosols was related to particle size and collecting fluid composition. Collection efficiencies, compared to the AGI-30, were 103% for 1.8 and 4.0 µm particles and 70% for 0.9 µm particles. Collection of 0.9 µm diameter particles improved with incorporation of 0.06% Tween 80 into the collecting fluid; tissue culture medium was better for recovery of the larger spore particles.

In an effort to improve the "simple liquid scrubber", White, et al. (1975) constructed a stainless steel large-volume air sampler. This cyclone scrubber, referred to as the Defense Research Establishment Suffield (DRES) — modified sampler, sampled at 0.95 m^3/min into a collecting fluid sprayed at 1-2 ml/min. Some of the modifications in the DRES sampler included: (1) redesign of a portion of the transition piece to collect all fluid; (2) facilitation of fluid collection by pressure drop between the sample outlet tube and blower (in place of a peristalic pump); (3) collection of fluid at a constant rate provided with a screw-driven syringe rather than a peristalic pump; (4) addition of a device for metered replacement of water lost by evaporation; and (5) addition of a removable preimpactor for discrimination of 10 µm MMD particles.

The DRES sampler was most efficient for collecting large particles. Evaluation of the device using vegetative bacterial aerosols verified the importance of collecting fluid selection for maximizing collection efficiency. The 0.06% Tween 80 solution used by Buchanan, et al. (1972) for *B. subtilis* var. *niger* spore collection, for example, was found to be toxic to *S. marcescens* and *E. coli* collected in AGI-30 reference samplers, but not in the DRES-modified sampler, which had an impaction velocity of about one-sixth the speed of sound. Aerosols containing spores with 3 µm MMD were collected with similar efficiency (74% to 86%) with all seven collecting fluids, while recovery of similar sized *S. marcescens*, *E. coli* and *A. aerogenes* aerosols were more affected by the collecting fluid.

F. Impaction

a. Conventional

When an obstacle is introduced into a flowing aerosol, the smaller particles follow the gas flow lines around the obstacle, whereas the large particles, owing to their greater inertia, are unable to change their direction and are intercepted by the obstacle. This phenomenon of impaction has been discussed extensively (Balzer, 1972; Ranz and Wong, 1952; May, 1945). Particle size, density, gas velocity, and target size are the primary factors influencing the efficiency of collection. Impaction of spherical particles on an obstacle is a function of impaction parameter K (McCormak and Hillard, 1970; May and Clifford, 1967), which may be defined as:

$$K = \frac{C \rho_p D_p^2 U}{18 \eta L}$$

where

K = impaction parameter — Stokes number

C = Cunningham slip correction factor

ρp = density of particle

U = characteristic velocity of the flow

L = characteristic length of obstacle

η = coefficient of fluid viscosity

An efficiency of impaction can be calculated by considering a cross section area of flow at a point sufficiently upstream for all particles contained therein to be flowing in a straight line toward the target. The envelope of trajectories that just graze the target contains all particles that may theoretically be removed by impaction (Hidy and Brock, 1971; Walton and Woodcock, 1960). Collision of particle with the obstacle is not always followed by adhesion or sticking. Hence the efficiency has to be corrected by the sticking coefficient (Dennis, 1976).

The samplers which use impaction as the mechanism for collecting are most efficient for larger particles. Smaller particles elude impaction, unless accelerated to very high velocities, but then the chances are much greater that the deposit will be swept away (Rosinski and Nagamoto, 1961).

May's description of the cascade impactor (1945) made a significant contribution to the application of impaction concepts to ambient particulate aerosol sampling. This device consisted of a system of four jets and slides in series. The progessively smaller jets resulted in increased air velocity at each level, permitting collection of progressively finer particles. Special emphasis was given to portability, ease of operation, and accuracy of sample collection. While recognizing that true isokinetic sampling could not be achieved in hold operations for all wind conditions, May designed the cascade impactor with an air inlet velocity of 3.5 m/sec (8 mph) with tolerable isokinetic deviation to sample in winds of 4-12 mph. A windvane, for continual sampling into the wind, and a critical orifice for maintenance of the desired air flow rate of 17.5 liter/min was included in the design. For still air sampling, a vertically situated adapter was recommended. This sampler was widely used in various air pollution studies.

The cascade impactor is used as a sampler but its principle use is as a particle size analyzer (Ranz and Wong, 1952; Andersen, 1958; May, 1945). Sonkin (1950) used a 5-stage cascade impactor for determining the concentration and size distribution of bacterial aerosols, and Harstad (1965) applied the sampler for collection and sizing of T-1 phage aerosol. As reported by Rao and Whitby (1977), the collection efficiency of the sampler is related to the adhesiveness of the collection surface. Patterson, et al. (1978) showed that with certain collection substrates the impaction efficiency of the impactor could be as much as 20% greater for charged than for uncharged particles.

Hill and Cox (1959) compared slit, slit with membrane filter impacting medium, four and six stage Andersen, and Porton all-glass impinger samplers for detection of airborne bacterial spores. Since air sampling rates were not recorded, a quantitative sampler comparison is not possible. The authors considered the performance of the Andersen samplers superior to the other devices.

Mitchell and Pilcher (1959) designed and calibrated an improved cascade impactor (Battelle cascade impactor) consisting of six circular particle size-discriminating jets and a final filter. They empirically optimized the effect of jet-to-slide clearance and wall-to-slide distance in the final design. A multijet round cascade impactor was described by Cohen and Montan (1967) who reported that particle size distribution data showed a larger standard deviation than that observed by microscopic analyses. Some of the problems associated with the device were attributed to inaccurately machined holes.

duBuy and Crisp (1944) described a sieve-type sampler that impacted airborne particles onto an agar plate with the sampling efficiencies being 43 to 73%. Gould (1970) reported recoveries of 60% of 1 to 20 µm particles with a modified sieve sampler in a tissue transplant facility. The importance of designing such a sampler with a maximum number of holes was discussed by May (1973). The total area of an agar plate on which particles are impacted increases and the impaction velocity decreases as the cube root of the number of holes.

A multistaged impactor was designed by Andersen (1958) to permit aerodynamic particle sizing by impaction onto the surface of staged agar-containing plates at increasing velocities. The Andersen sampler consisted of six sieves of decreasing jet diameters. Because the device operated on the basis of inertial impaction, the critical agar-to-jet distance required use of specially constructed petri dishes containing 27 ml agar-based medium with a sampling rate of 28.3 liter/min (1 cfm). Widespread acceptance of this sampler led to its being recommended as a standard sampler for collection of airborne microorganism-containing particles (Brachman, et al. 1964).

Although the Andersen sampler was suitable for many applications, various modifications or adaptations were proposed to improve or adapt the sampler to special needs. May (1964) reported that the efficiency of sampling larger particle aerosols could be increased by adding a top stage to the apparatus and by redesigning the holes. Lidwell and Nobel (1965) designed a prestage for the Andersen sampler to sample larger particles.

Efforts to apply the Andersen sampler for assay of total airborne microorganisms, rather than microorganism-containing particles, by washing the particle-laden agar surface also resulted in various sampling surface modifications. As noted by May (1966), microorganism recovery from particles impacted on the agar surface is reduced by the ineffectiveness of agar washing. Consequently, many surfaces have been utilized to enhance the removal of microorganisms for assay. Jensen (1964) found the Andersen sampler to be more efficient than the AGI for virus sampling when the aerosol was impacted onto skim milk-coated agar surfaces, which were subsequently washed and assayed. Plain agar was only one-half as efficient as skim-milk-coated agar surfaces for such assays. Gelatin layered on agar has been used for enterovirus assay following gelatin liquification at 37°C (Couch, et al. 1965; Gerone, et al. 1966).

Guerin and Mitchell (1964) used 5 ml per plate of tissue culture medium containing 3% gelatin for enterovirus collection in Andersen samplers. It is important to note that the Andersen sampler was designed for operation with 27 ml of medium and that alteration of this volume affects the aerodynamic properties and, consequently, the collection efficiency of each sampler stage. Thomas (1970) suggested that direct liquification, washing methods and direct sampling onto cells all had severe limitations and that a mixture of equal parts of saturated sucrose solution and glycerol with 0.1% of 10% bovine serum albumin (S.G.B. mixture) was superior to other methods of sampling virus aerosols in modified Andersen samplers. This method has a particular advantage, namely, it permits the determination of the particle size range of an aerosol and the direct viral assay by plaquing techniques.

The Andersen sampler is also limited by the volumes of air which may be sampled within a specific period without significant viability losses as a result of drying the upper stages at the site of impaction. This sampler was reported not to be suitable for sampling air containing a very low concentration of viable particles. To extend the sampling time of the Andersen and other agar impaction-type samplers, May (1969) reduced agar drying by application of a monolayer of oxyethylene docosanol (OED). This monomolecular film retarded evaporation without affecting the viability of collected organisms. The OED was originally used as an evaporation retardant to prevent heat loss from rice fields (Mihara, 1962). Since its introduction into the field of aerobiology, OED has been used extensively in slit and Andersen samplers (Andersson, et al. 1973).

Although the Andersen sampler was first described for use with agar plates for bacterial aerosol studies, the device has been modified for non-viable applications. Such a modification (Andersen, 1966) enabled the collection and aerodynamic sizing of non-viable airborne particulates.

A plastic disposable two-stage Andersen sampler was described by Turner and Hill (1975) and was reported to be highly efficient for recovery of aerosols in the 0.8 to 10.5 µm range. Other investigators, however, found that this device recovered significantly fewer airborne microorganism-containing particles than the multistaged Andersen sampler (Curtis, et al. 1978).

A rotating agar drum sampler, the monitor, was described by Andersen and Andersen (1962) for collection of bacterial aerosols over a relatively long time period. Collection was achieved by inertial impaction onto the agar drum following particle acceleration through a round jet. This device could be operated for relatively long periods of time, up to 48 hr, but the sample collection rate of 0.5 to 3 liter/min limits its usefulness when greater sensitivity is required. A sampler for airborne bacterial monitoring for relatively long time periods, operating on the impaction principle, was described by Reckzeh and Dontenwill (1974). This device could be programmed to take samples automatically for up to 24 hr.

Impaction from a moving airstream by means of a funnel device was described by Hollaender and DallaValle (1939). This device was reported to collect 80% of 2 µm B. subtilis spores in experimental studies. The efficiency of such a sampler depended upon the distance between the funnel outlet and the agar-containing petri dish (Bourdillon, et al. 1941). This device was regarded as being useful for larger particles.

A slit sampler for collection and enumeration of airborne bacteria was described by Bourdillon, et al. (1941). Air was drawn through a 0.25 x 27.5 x 3.0 mm slit and airborne particles were impacted onto the surface of a rotating agar plate. At an air sampling rate of 28.3 liter/min (1ft^3/min) and 27.9 cm (11 in.) water vacuum, the air was accelerated to 82 m/sec (270 ft/sec) and the sampling efficiency was found to be greatest when the slit-to-plate distance was 2 mm. Drying of agar could increase the slit-to-agar distance, resulting in a reduction in particle impaction efficiency. This drying may be substantially reduced by the application of a substance such as OED to the agar surface (May, 1969).

Because particles were impacted on a slowly rotating agar plate, a relationship between time and microbial aerosol concentration could be established with the slit sampler. Kuehne and Decker (1957) showed that aerosols of S.

marcescens could be sampled continuously for up to 12 hr when using a slit sampler developed by Decker and Wilson (1954). Decreased speed of plate rotation, which permitted a larger sample of air to be collected in a single plate, resulted in decreased bacterial recovery percentages. Using 3% agar, 1 hr sampling did not affect S. marcescens recovery. Longer sampling periods resulted in decreased recoveries, but these recoveries increased when the agar concentration decreased to 1.5%. Once the organisms were collected on the agar surface, continued sampling did not substantially affect viability.

In order to extend the duration of sampling without loss in microbial recovery efficiency, Decker, et al. (1958) developed a slit-incubator sampler. This device contained an incubation chamber into which an agar-filled rectangular culture tray was pushed after passing beneath the slit at an air velocity of 34 m/sec (110 ft/sec). The highest recovery of S. marcescens aerosols (74% when compared to the Decker and Wilson slit sampler) was found when the time for passage of the entire agar tray was set at 7.5 hr. Although this device enabled longer sampling periods, the lower collection efficiency should make it practical only for special applications for which the original sampler is not suited.

Another slit sampler designed for collecting air samples over a relatively long time period was described by Brown (1973). This device consisted of an outsize sampler with a 62 cm(2 ft) diameter culture plate onto which airborne particles were deposited along long (approximately 2m) spiral path, thus utilizing the entire available surface. The air sampling rate was 20 liter/min and, using a switching arrangement, samples were taken every 30 min for 24 hr. For maximum collection efficiency, the slit was 29 mm long and 0.25 mm wide with a slit-to-agar distance of 1 mm. Preparation of the agar in the large collecting plate required a special leveling device.

A slit sampler most appropriate for outdoor use was described by Andersson, et al. (1973). The FOA slit sampler was constructed to sample into the wind with an inlet velocity of 5 m/sec at a sampling rate of 20 liter/min through a 50 x 0.2 mm slit, achieving an impaction velocity of 41.7 m/sec and causing particle impaction onto a standard 15 cm plastic plate. To reduce desiccation, OED was added to the agar medium surface. The horizontal sample inlet on the FOA sampler reportedly gave a tolerable deviation from isokinetic sampling at wind speeds up to 10 m/sec (May, 1967).

The described slit samplers were not very useful when high sensitivity was required for detecting very low concentrations of microbial aerosols. In an attempt to increase this sensitivity, a Casella large model slit sampler was described which would operate at a flow rate up to 700 liter/min (May, 1973). The design included 4 slits located 90° apart and directly above a rotating petri dish containing agar. This device, however, permitted neither time nor particle size discrimination.

Since the slit sampler has been widely used it has been compared to various other sampling devices. The Reynier slit sampler, for example, was found to recover significantly more bacterial particles than would be recovered with membrane filter samplers (Fields, et al. 1974). It was suggested that some larger particles may break up during slit sampler collection, thus giving higher counts, but it is likely that desiccation on membrane filters contributed substantially to these findings. Berche, et al. (1978) found bacterial aerosol recoveries of the MK II slit sampler (Cassella, London) and the RB type MI (Joubert, Lyon) to be similar, sampling at 0.70 and 0.75 m^3/min, respectively.

Comparative studies with the slit and all-glass impinger samplers were reported by Ehrlich, et al. (1966). Since the slit sampler collects particles containing microorganism, it was necessary to wash the particles from agar plate surfaces before assay for total microorgamisms, as enumerated from AGI samplers. Using this agar washing method, it was found that the slit sampler was only about 50% as efficient as the AGI-30 for recovery of S. marcescens or B. subtilis var. niger spore aerosols. As noted by May (1966), however, impacted microorgamisms cannot be easily washed from agar surfaces. Vlodovets, et al. (1960) attributed the low recovery of airborne influenza virus from slit samplers after washing to partial virus retention on the agar surface. DuBuy, et al. (1945) noted that impaction devices do not give a time count of bacteria numbers, with bacteria-containing clumps having an average of 10 organisms.

To minimize losses of microorganisms due to washing from agar surfaces, a number of investigators have reported use of techniques such as those used with Andersen samplers. Dahlgren, et al. (1961) and Kuehne and Gochenour (1961) collected airborne T-3 phage and Venezuelan equine encephalitis virus onto a gelatin surface, followed by liquification and assay. Use of gelatin in slit samplers, however, was reported to cause sampling difficulties. Ehrlich, et al. (1966) reported that the gelatin could not be solidified unless ice packs were used with the sampler. These ice packs then produced a condensate that leaked into the sampler resulting in erroneous data. Such problems were avoided by the use of a soluble adhesive surface described by Thomas (1970). Using a slit sampler and an adhesive surface, Thomas (1974) reported the detection of smallpox virus aerosols from an isolation hospital.

Goldberg (1971) reported a syringe-slit sampling technique for direct assay of aerosol samples which reduced the labor required when assaying impinger samples. The aerosol was sampled from a chamber with a syringe which was, in turn, inserted into a fixture on a slit sampler containing an agar medium plate. By depressing the syringe plunger and activating the slit sampler vacuum, the collected sample was plated directly onto the agar medium. It was suggested by Goldberg and Scechmeister (1951) that a "slit index" associated with a given set of slit sampling parameters could be used to estimate the particle size of microbial aerosols.

Sawyer and Walton (1950) described a conifuge for classifying airborne particles on a rotating slide according to their settling velocities. The rotorod sampler collects particles greater than 1 µm at 41 liter/min by impaction onto rotating rod-mounted slides (Am. Conf. Gov. Ind. Hyg., 1972). This device utilizes moving collection surfaces and consists of a constant speed motor and two collector rods rotated by the motor. The collecting areas of the rods are coated with an adhesive surface to reduce bounce-off of the particles. Size separation can be accomplished by using different sized rods. Collection efficiency is independent of wind speed although highly dependent upon particle size. The sampler, which may be operated on AC or DC current, is portable and rugged. The rotorod has been used primarily for airborne pollen studies, but has been applied for airborne microorganism detection. Evaluation of the rotorod for microbial aerosol detection demonstrated its usefulness in a hospital environment and its comparability to settling plates for bacterial recoveries (Kittingen and Mauseth, 1967).

Recognizing a need for a large volume microbial air sampler which permitted particle size discrimination while being relatively simple and robust, May (1973) described the Pagoda sampler. The sampler was capable of sampling at 1000 liter/min while impacting particles 1 to 5 µm, 5 to 18 µm and greater than 18 µm on an agar surface. May reported sampling for up to 1 hr when OED-coated agar was used. Such a device may be very appropriate for detecting and sizing bacteria-containing particles when present in relatively low concentrations. A major disadvantage of this sampler is that it is not readily applicable for enumeration of total bacteria or viruses. A liquid medium would be most suitable for such an application.

Mammeralla (1966) described a multiple funnel sampler containing four funnels of 1 to 4 mm on a metallic cylinder with a funnel opening to agar collection plate surface distance of 2 mm. Particle size discrimination is based upon the funnel opening diameter and consequent impaction velocities. The sampler has the unique capability of collecting particles in four size ranges on the same agar plate.

A sampler for collecting bacteria in particles greater than 10 µm diameter was described by Mammeralla (1965). This device, the cascade vault sampler, consisted of a cylindrical base containing an agar-filled petri dish with a cover containing a series of three "steps" arranged at right angles to the direction of the airflow, with each step higher than the last. As the air was impacted onto the vertical walls of the step structure, a portion of the airborne particles were lost from the airstream and deposited, by gravitational settling, onto the agar surface. A major limitation of this device is that it is suitable only for collection of relatively large particles.

An air sampler for field use without electrical current was described by Vasil'yev (1957). A four-blade fan was operated by hand, through a series of gears, to pull air through a hollow truncated cone. Particles were collected by impaction onto a medium-containing petri dish.

A single stage impactor was described by Malligo and Idoine (1964). This device was designed for particle size selection when used with an AGI sampler. By employing the concept of inertial impaction (Ranz and Wong 1952), the single stage impactor collected particles greater than a given size onto a metal disc. Smaller particles were then permitted to be collected by the AGI. Larson, et al. (1973) compared the single stage impaction device to the multistage liquid impinger and concluded that either device may be used for sampling and sizing microbial aerosols.

b. Virtual

Efforts to minimize particle bounce-off losses frequently encountered in conventional impactor-type samplers resulted in application of the virtual impaction concept for aerosol collection. The particles are impacted into a collecting nozzle containing a slow-moving airstream rather than onto a conventional solid surface. This concept was employed by Hounam and Sherwood (1965) in the design of the cascade centripeter which had particle size cutoff of 12, 3.5 and 1.2 µm. Wall losses were, however, reported to be up to 40% for the second and third stages. Subsequently, the dichotomous virtual impactor was refined for separation of particles into two aerodynamic size ranges (Dzubay, et al. 1977; Dzubay and Stevens, 1975; Loo, et al. 1976). Aerodynamic sizes are related to the size, shape, and density of particles as they affect aerosol behavior. Stevens and Dzubay (1978) described the development and evaluation of manual and automated dichotomous samplers for collection of $\leq$ 3.5 µm and > 3.5 µm particulates onto filters for measurement of mass and chemical composition. The sampling rate was 17 liter/min with approximately 10% of the air passing through the coarse particle filter and the remaining air through the fine particle filter. McFarland, et al. (1978) described particle collection characteristics of the single stage dichotomous sampler using a 14 liter/min flow rate under various conditions. They emphasized the advantages of a single stage over a two-stage dichotomous sampler. After comparison of the Sierra Instruments, Inc. dichotomous virtual impactor with a cyclone, John, et al. (1978) recommended an air sampling rate of 10.7 liter/min for the former device. Application of the dichotomous virtual impactor for ambient viable microbial air sampling is limited by the low air flow rate and by the filter system for particle entrapment. Higher air flow rates are required for detection of low-level microbial aerosols (Decker, et al. 1969) and viability losses due to desiccation during filtration have been reported.

In a comparison study of eleven different nonviable aerosol samplers it was found that automatic dichotomous samplers showed the greatest precision for the fine fraction (2.4 µm) aerosol particles (Camp, et al. 1978).

To achieve relatively long term, remote, large-volume sampling for biological aerosol detection, a device employing the concepts of virtual impaction and subcritical impingement was developed for the U.S. Army. The XM2 Biological Sampler was designed to operate continuously with daily servicing (Environmental Research Corporation, 1975). Airborne particles of 2 to 12 µm aerodynamic size range were concentrated from 1050 liter/min to 15 liter/min air flow, collected by bubbling through a continuously flowing sampling medium and either discarded or saved at 4°C (Environmental Research Corporation, 1974). The saver containers were sized to receive up to three 10 min samples. That is, a maximum air sample size of 30 m^3 could be remotely obtained daily.

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